Extractive Fermentation Systems for Organic Acids Production

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Basic characteristics of on-line extractive fermentation of organic acids were examined using a general model for the integrated process in order to illustrate the effects of various process parameters and operational modes on system performance. The strong pH dependence of both the fermentation kinetics and the extraction efficiency has been outlined and taken into account by inclusion of a model equation which predicts the pH profile. Simulation results using data from butyric acid fermentations show that our complete model system is adequate to evaluate different operational modes of the process, including simple batch fermentation, batch or fed-batch fermentation with extractive recycle, and continuous fermentation with extractive recycle. In the case where a second undesired acidic byproduct is also produced, our model predictions suggest that on-line extractive fermentation using a suitable solvent results in an effective gross separation of the two acids.

On a examiné les caractéristiques de base de la fermentation extractive continue d'acides organiques à l'aide d'un modèle général pour le procédé intégré afin de décrire les effets de divers paramètres de procédés et modes opérationnels sur la performance du système. La forte dépendance au pH de la cinétique de fermentation et du rendement de l'extraction est décrite puis prise en considération par l'inclusion d'une équation de modèle qui prédit le profil de pH. Les résultats de la simulation qui utilisent des données de fermentation de l'acide butyrique montrent que notre système de modèle complet convient pour évaluer les différents modes opérationnels du procédé, dont la fermentation discontinue simple, la fermentation discontinue ou à alimentation discontinue avec recyclage extractif et la fermentation continue avec recyclage extractif. Dans le cas où un deuxième produit acidique non désiré est également produit, nos prédictions de modèle suggèrent que la fermentation extractive continue utilisant un solvant adéquat permet une séparation brute efficace des deux acides.

Keywords: organic acids, extractive fermentation, computer simulation, biocompatible solvents.

Organic acids are large volume industrial and food grade chemicals with an annual US market alone exceeding 5 billion pounds. They can be produced through chemical synthesis or from direct fermentation using carbohydrates as substrates. The structure and properties of these acids may vary significantly with the type of microorganisms and the biochemical pathway through which they are produced (Atkinson and Mavituna, 1983; Stanier et al. 1976). However, all of them are characterized as organic acids with pK_a values lying between 1.0 and 8.0. One can classify these fermentation products distinguishing four general categories based on the major biochemical pathway followed in their production. The first type of organic acids (Type I) are those produced in aerobic conditions through the citric acid cycle (tricarboxylic acid pathway). The second type of fermentation organic acids (Type II) consists of those produced under anaerobic conditions, mainly through the metabolic pathway of butyric acid bacteria. These *Clostridia* can ferment many sugars to produce a variety of solvents and low molar mass organic acids, such as acetic, butyric, lactic and formic acids. Type III organic acids consists of pharmaceutically important compounds such as antibiotics, vitamins and amino acids. These compounds are produced through more complicated biochemical pathways by specific types of microorganisms. The fourth category includes fatty acids produced through hydrolysis. Some organic acids can also be produced through oxidative cleavage of synthetic organic compounds, such as toluene or benzene, using oxygenase enzymes. Various representative organic acids of Types I and III are given in Table 1 along with their pK_a values.

During the last two decades there has been a resurgence of interest in large-volume production of chemicals by fermentation. The current economic impact of these products is still limited, in part due to the difficulties of product recovery. Consequently, for fermentation based acids to penetrate the organic chemical market, substantial improvements in recovery technology are needed (Lockwood, 1979; Kertes and King, 1986). This is especially true for Types I and II organic acids, where more economically competitive synthetic sources are available.

TABLE 1
Fermentation Organic Acids and Their pK_a Values

Acid	Type	pK_a (25°C)
Pyruvic acid	I	2.49
Citric acid	I	3.13, 4.76, 6.40
Isocitric acid	I	3.29, 4.71, 6.40
Succinic acid	I	4.20, 5.64
Fumaric acid	I	3.02, 4.38
Malic acid	I	3.22, 4.70
Oxalic acid	I	1.23, 4.19
Maleic acid	I	1.93, 6.14
Itaconic acid	I	3.65, 5.13
Tartaric acid	I	3.01, 4.38
Propanoic acid	I	4.85
Lactic acid	I	3.86
Acetic acid	II	4.75
Butyric acid	II	4.81
Formic acid	II	3.75 (20°C)
Lincomycin	III	7.65
Flurbiprofen	III	6.30

Type I: from tricarboxylic acid pathway; aerobic
Type II: products of anaerobic metabolism (mainly butyric acid bacteria)
Type III: pharmaceutically important compounds

Extractive fermentation

Extractive fermentation, the combination of the bioreaction with liquid–liquid extraction in a unified process, is very promising for improved production of a wide variety of compounds, ranging from specialty chemicals such as antibiotics

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and vitamins to commodity chemicals such as alcohols, organic acids, and solvents. This process is particularly attractive for bioreactions where cell growth and/or product formation are regulated by the product or by other undesirable cellular metabolites (Minier and Goma, 1982; Wang, 1983; Roffler et al., 1984; Fournier, 1986; Bar and Gainer, 1987). Since solvent extraction is applicable to the separation of many organic acids, on-line extractive fermentation can potentially be used in the production of these acids. Cell growth (and consequently the rate of product formation) is usually inhibited by the extreme pH in the fermentation broth resulting from the release of the acidic products (Pacaud et al., 1986; Wang and Wang, 1984). Using an appropriate biocompatible extractant, on-line extractive fermentation for these bioreactions can be expected to improve system performance by reducing product inhibition and toxicity and by isolating the various acidic products.

An appropriate solvent for on-line extractive fermentation should satisfy an additional constraint: it must be non-toxic to the microorganisms. Unfortunately, solvents that efficiently extract the desired product are usually highly toxic to microorganisms (Evans and Wang, 1987). Often an extractant with a high distribution coefficient for the product is chemically similar to the product. However, chemically similar compounds may have similar mechanisms of microbial toxicity, so the extractant as well as the product may be toxic to the cells.

Only a few suitable organic liquid extractants exist at present. We have been investigating the possible use of mixed extractants (a combination of toxic and non-toxic extractants with corresponding high and low distribution coefficients for the product) for on-line extraction (Evans and Wang, 1987). Initial success was achieved for butanol and butyric acid fermentations (Evans and Wang, 1988, 1990).

On-line extraction of organic acids

The model bioreaction chosen for this study was the butyric acid fermentation in which acetate and butyrate are the major acidic products. Both products are produced in comparable concentrations, and thus effective separation of the more valuable one (butyrate) is very important in reducing the total cost of the process. Also, since both butyrate and acetate inhibit cell growth, product removal during the fermentation will definitely improve productivity. We consider only the on-line extraction of the non-ionized forms of butyrate and acetate by an immiscible, biocompatible organic solvent. Extractive fermentation has been applied with satisfactory results to several other fermentations, including organic acid fermentations, by reducing product inhibition and increasing process productivity (Datta 1981; Roffler et al., 1984; Bar and Gainer, 1987; Evans and Wang, 1990).

A general scheme for an idealized continuous on-line extraction process is given in Figure 1. Whole broth or filtered supernatant and solvent can be contacted either in the fermenter or in an external extraction vessel. Contact of broth and solvent in the fermentation vessel results in a limited interfacial area between the two phases and low rates of mass transfer. Thus, product can accumulate in the aqueous phase since its formation rate may be much higher than its transfer rate to the organic phase (Diaz, 1988; Roffler, 1986). Therefore, we assume contact of broth and solvent to take place in a separate extraction vessel. Since the type of extraction equipment is a separate design problem, we chose a single ideal stage contactor-phase separator for initial evaluation here. The recycle stream leaving the fermenter contacts the free solvent in the extraction vessel. The aqueous phase leaving the extractor returns to the fermenter, while the organic extract is processed further for product recovery and free solvent regeneration. Finally, the fermenter is equipped with a feed line for additional supply of substrate and nutrients and a bleed line for the disposal of used cells and medium.

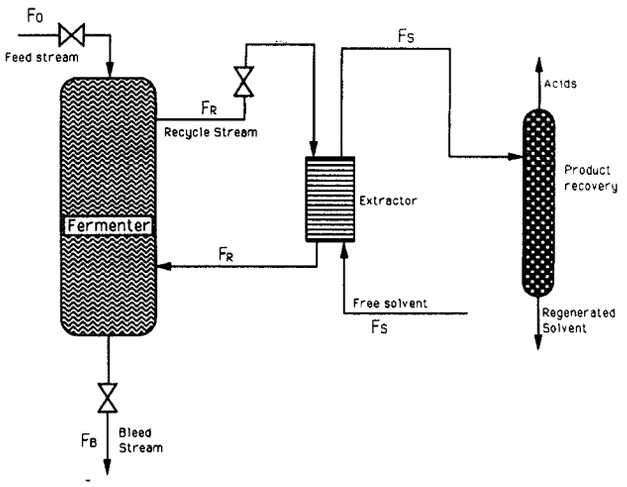

Figure 1 — A schematic diagram of the continuous on-line extractive fermentation.

In addition to butyrate, the organic solvent will also extract a significant amount of acetate. Isolation of butyrate from the fermentation broth depends on the extract-to-raffinate ratio, on the nature of the organic solvent and on the distribution coefficients of the two acids. The ratio of the distribution coefficients for butyrate and acetate determines the effectiveness of the separation. Under certain conditions, the ratio of the distribution coefficients may change dramatically with the pH of the extractor's aqueous phase, and the separation of these two similar compounds may be greatly enhanced or reduced (Robinson and Cha, 1985). Thus, a valid model for pH prediction in organic acid fermentation broths, such as the one previously developed (Hatzinikolaou and Wang, 1991; Pons et al., 1990), is of great importance in designing the entire extractive fermentation process.

Oleyl alcohol with varying amounts of decanol (Evans and Wang, 1987) was used as the model extracant in this work. This organic mixture is chosen because it is non-toxic to *Clostridia* spp., and because it has a high capacity for butyrate. Furthermore, there are available experimental data on the distribution coefficients of butyrate and acetate from aqueous solutions (Lemon and Wang, 1988). The actual distribution coefficient of butyrate ($K_{D\text{-}but}$) is defined as:

$$K_{D\text{-}but} = [ButH]_o / ([ButH]_w + [But^-]_w) \quad \ldots\ldots \quad (1)$$

where $[ButH]_o$ and $[ButH]_w$ are the concentrations of the non-ionized form of the acid in the organic and aqueous phase, respectively, and $[But^-]_w$ is the concentration of the ionized form in the aqueous phase. In the definition of Equation (1), it is implied that But^- is not soluble in the organic solvent. Substituting $[But^-]_w$ from the definition of the dissociation constant, K_{but}, gives:

$$K_{D\text{-}but} = [ButH]_o / \{ [ButH]_w [1 + (K_{but} / [H^+]_w)] \} \quad (2)$$

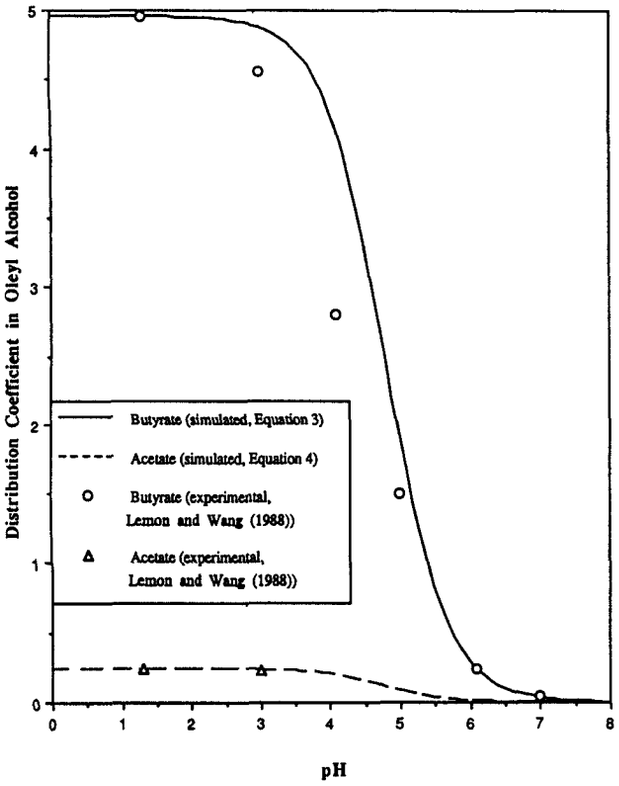

Figure 2 — Distribution coefficients of acetate and butyrate versus pH (Solvent: oleyl alcohol).

The ratio $[ButH]_0/[ButH]_w$ is defined as the intrinsic distribution coefficient (Robinson and Cha, 1985), $K^*_{D\text{-}but}$, which applies at very low pH values, where all the acid is present in the non-ionized form. Assuming that this ratio is not affected by the presence of But^-, Equation (2) may be written as:

$$K_{D\text{-}but} = K^*_{D\text{-}but}/[1 + (K_{but}/[H^+]_w)]$$

or

$$K_{D\text{-}but} = K^*_{D\text{-}but}/[1 + 10^{\text{pH-}pK_{but}}] \quad \ldots\ldots\ldots\ldots\ldots \quad (3)$$

Similarly, for acetate we may write

$$K_{D\text{-}ac} = K^*_{D\text{-}ac}/[1 + 10^{\text{pH-}pK_{ac}}] \quad \ldots\ldots\ldots\ldots\ldots \quad (4)$$

With the values of the intrinsic distribution coefficients known, we can determine the actual distribution coefficient for each acid, at every pH value of the extractor's aqueous phase. The values of $K^*_{D\text{-}but}$ and $K^*_{D\text{-}ac}$ for oleyl alcohol at pH 1.0 have been previously determined and are 4.95 and 0.24, respectively (Lemon and Wang, 1988). Inserting these values in Equations (3) and (4) we plotted $K_{D\text{-}but}$ and $K_{D\text{-}ac}$ as a function of the pH in the extractor's aqueous phase (Figure 2). In this figure, experimental values of the distribution coefficients of the two acids in oleyl alcohol at specific pH values are also plotted (Lemon and Wang, 1988). It seems that both equations can be used to predict the K_D's of the two acids as long as the pH of the aqueous phase is known. From Figure 2 is also clear that extraction efficiency is favoured at low pH values where the distribution coefficients are high. Both K_D's tend to zero at high pH values, since at this pH range all the acids are in the non-extractable ionized

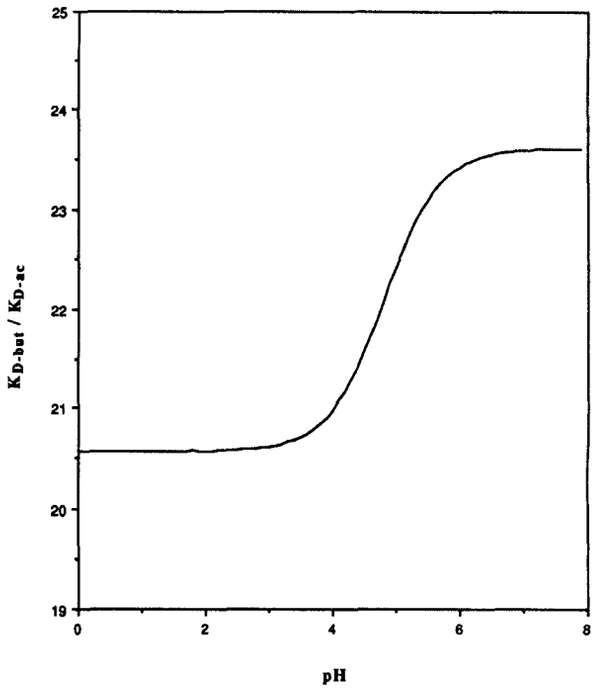

Figure 3 — Ratio of the distribution coefficients of butyrate and acetate in oleyl alcohol.

form. Their ratio however, $K_{D\text{-}but}/K_{D\text{-}ac}$, increases with the pH (Figure 3). Since this ratio determines the effectiveness of the separation among the two acids, we may conclude that the latter is improving by increasing the pH.

The biochemical kinetic model

There are many bacteria capable of utilizing water-soluble sugars (e.g. glucose) to produce organic acids. In modeling the pH profile during fermentation and on-line extraction, it is essential to know the kinetics of cell growth, substrate utilization, and product formation. The parameters of any fermentation model must be determined from batch or continuous fermentation data for each microorganism and set of growth conditions. The process is strongly pH dependent and there is evidence that the products exhibit various inhibition effects on the cell growth (Roffler, 1986; Pacaud et al., 1986; Wang and Wang, 1984). In this work the focus is on the butyric acid fermentation as a model system. Butyric acid bacteria such as *Clostridia* spp. can anaerobically ferment a variety of sugars to produce a number of organic solvents (butanol, acetone, ethanol, etc.) and/or carboxylic acids (acetate, butyrate, lactate and formic acids). In modeling the fermentation kinetics we shall use the following assumptions:

- only two basic metabolites (acetate and butyrate) are produced.
- other toxic metabolites are absent (only the main products inhibit cell growth).

Several researchers have suggested that the fermentation products substantially inhibit cell growth. Thus, the traditional Monod growth model should be modified to take this into account:

$$R_c = [\mu_{max} S/(K_s + S)]\,[I_{ac}/(I_{ac} + [ac])]$$

$$[I_{but}/(I_{but} + [but])]X - k_d X \quad \ldots\ldots\ldots\ldots\ldots \quad (5)$$

The values of μ_{max}, K_s, and k_d vary with the medium composition, the temperature, the strain of organism, etc.

I_{ac} and I_{but} are inhibition constants for acetate and butyrate, respectively.

The pH drop during fermentation without pH control will affect the growth of the microorganisms. This effect is generally described through the well-known bell shaped curve of μ_{max} vs pH. There has been no attempt in the literature to model this effect. In addition, the question of whether growth inhibition is mainly due to the release of the acid(s) or to the pH value itself is still unsettled. Thus, in Equation (5) we are assuming that during the fermentation the pH changes by 1 to 2 units, but that there is no significant effect of pH on μ_{max}. Also we may assert, that since the pH drop in the broth is caused by the release of organic acids, the acid inhibition pattern introduced in Equation (5) indirectly accounts for the pH inhibition on cell growth.

For product formation, a mixed growth kinetic model of the Luedeking–Piret type was used (Bailey and Ollis, 1986):

$$R_{ac} = a_a R_c + b_a X \quad \ldots\ldots (6)$$

and

$$R_{but} = a_b R_c + b_b X \quad \ldots\ldots (7)$$

where $a_{a,b}$ = mmol acids/g cells and $b_{a,b}$ = mmol acid/g cells · h.

Finally, the rate of substrate utilization may be written as:

$$R_s = (1/Y_{x/s})\, R_c + (1/Y_{ac/s})\, R_{ac} + (1/Y_{but/s})\, R_{but} \quad (8)$$

where the Y's are the substrate yield coefficients for biomass, acetate and butyrate, respectively. Using the above rate expressions in Equation (5) to (8), we can develop the differential equations for batch fermentation of butyric acid bacteria:

$$dX/dt = R_c \quad \ldots\ldots (9)$$

$$-dS/dt = R_s \quad \ldots\ldots (10)$$

$$d[ac]/dt = R_{ac} \quad \ldots\ldots (11)$$

$$d[but]/dt = R_{but} \quad \ldots\ldots (12)$$

Equations (9)–(12) can be used in order to determine the values of the kinetic constants through batch fermentation experimental data. In this work the values of these constants [except for $Y_{ac/s}$ and $Y_{but/s}$, which can be determined from the biochemical pathway (Papoutsakis, 1984)] were determined numerically using simultaneous integration and nonlinear optimization techniques. The Simusolve software package (MTS, The University of Michigan) was used.

Modeling of the continuous on-line extraction

For the on-line extraction model we used the following assumptions.

— The fermenter is a well mixed reactor.
— The phases are immiscible.
— The two phases are always in equilbrium.
— Only acetate and butyrate are distributed among the two phases.
— The K_D's for the two products are independent.
— The K_D's are dependent on the pH of the aqueous phase (Equations 3 and 4).
— No volume change occurs on mixing.
— The extract and raffinate flowrates are constant.

Under these assumptions, and applying the appropriate mass balances for the general on-line extractive fermentation system of Figure 1, we obtain the differential equations that describe the variation of species in the fermenter and extractor with time (Table 2).

Equations (13) to (21) in Table 2 have to be integrated simultaneously using a fourth-order Runge Kutta method to give the concentrations of the various species in the fermenter and the extractor aqueous phase. From the definition of the distribution coefficient, K_D, the concentrations of the acids in the organic extract at each time step are given by:

$$[ac]_E^O = K_{D\text{-}ac}[ac]_E^A \quad \ldots\ldots (24)$$

$$[but]_E^O = K_{D\text{-}but}[but]_E^A \quad \ldots\ldots (25)$$

It is noted that the distribution coefficients of acetate and butyrate in the solvent are functions of the concentrations of acids in the extractor aqueous phase (since they are a function of pH), and thus their values are time dependent. Therefore, an iterative algorithm (that involves integration step adjusting) for the calculation of the distribution coefficients using a pH predicting model for the particular fermentation medium and conditions (Hatzinikolaou and Wang, 1991) had to be employed at each integration time step.

Computer simulations of the on-line extraction of organic acids

The model microorganism we used for our simulations is *Butyribacterium methylotrophicum*. The working pH range for this bacterium is 7.5 to 4.8, and its two major metabolic products are acetate and butyrate using glucose as the sole carbon-energy source. Using batch fermentation data for this microorganism (Shen et al., 1988), we determined the kinetic constants for growth, through nonlinear optimization routines using the Simusolve program and Equations (9)–(12). A summary of these values is shown in Table 3. The nonlinear fitting of the cell growth kinetic model is given in Figure 4.

The working fermenter volume was taken to be 10 L and the volume of the extractor was taken as 1 L. The objective function for the evaluation of the system's performance is the fermenter productivity of butyrate, defined as:

$$V = B_{tot}/t_f \quad \ldots\ldots (26)$$

But since there are two major products in this process, the purity of the extract is also important and has to be taken as an additional objective function. This parameter is defined as:

$$P = B_{ext}/(A_{ext} + B_{ext}) \quad \ldots\ldots (27)$$

The first process parameter effects evaluated are those of the recycle flowrate F_R and the solvent flowrate F_S. As shown in Figure 5, the predicted butyrate productivity strongly depends on F_R; it increases rapidly at recycle flowrates of between 0.0 and 0.3 fermenter volumes/h while

TABLE 2
Differential Equations Developed for Computer Simulation of Organic Acid Fermentations

The mass balances on the cells, substrate and products in the fermenter are:

Cells:

$$dX_R/\mathrm{dt} = [1/V_R]\,[F_0X_0 + F_R(X_E - X_R) - F_BX_R + R_cV_R - X_R(F_0 - F_B)] \quad (13)$$

Substrate:

$$dS_R/dt = [1/V_R]\,[F_0S_0 + F_R(S_E - S_R) - F_BS_R - R_sV_R - S_R(F_0 - F_B)] \quad (14)$$

Acetate:

$$d[ac]_R/dt = [1/V_R]\,[F_0[ac]_0 + F_R([ac]_E^A - [ac]_R) - F_B[ac]_R + R_{ac}V_R - [ac]_R(F_0 - F_B)] \quad (15)$$

Butyrate:

$$d[but]_R/dt = [1/V_R]\,[F_0[but]_0 + F_R([but]_E^A - [but]_R) - F_B[but]_R + R_{but}V_R - [but]_R(F_0 - F_B)] \quad (16)$$

Fermenter volume:

$$dV_R/dt = F_0 - F_B \quad (17)$$

The mass balances on the cells and substrate in the extractor, which involve only the aqueous phase, are:

Cells:

$$dX_E/dt = [1/V_E^A]\,[F_R(X_R - X_E) + R_CV_E^A] \quad (18)$$

Substrate:

$$dS_E/dt = [1/V_E^A]\,[F_R(S_R - S_E) - R_SV_E^A] \quad (19)$$

The material balance on acetate in the extractor is:

$$d(V_E^A[ac]_E^A)/dt + d(V_E^O\,[ac]_E^O)/dt = F_R[ac]_R + V_E^AR_{ac} - F_R\,[ac]_E^A - F_s[ac]_E^O$$

Substituting the equilibrium relationship $[ac]_E^O = K_{D-ac}\,[ac]_E^A$ into the above equation, the acetate concentration in the extractor aqueous phase is given by the following differential equation:

Acetate:

$$d[ac]_E^A/dt = [1/(V_E^A + K_{D\text{-}ac}\,V_E^O)]\,[F_R[ac]_R + V_E^AR_{ac} - (F_R + K_{D\text{-}ac}F_S)[ac]_E^A] \quad (20)$$

Similarly, the butyrate concentration in the extractor aqueous phase is given by the following equation:

Butyrate:

$$d[but]_E^A/dt = [1/(V_E^A + K_{D\text{-}but}\,V_E^O)]\,[F_R[but]_R + V_E^AR_{but} - (F_R + K_{D\text{-}but}F_S)[but]_E^A] \quad (21)$$

Extractor volume:

$$V_E^A = [F_R/(F_R + F_S)]\,V_E \quad (22)$$

$$V_E^O = [F_S/(F_R + F_S)]\,V_E \quad (23)$$

it remains practically constant at rates greater than 0.6 fermenter volumes/h. This result indicates the importance of butyrate removal from the broth, since the inhibition effect of the acid on cell growth (and consequently on the rate of product formation) is significantly reduced. The nearly constant productivity at high recycle flowrates can be attributed to the fact that solvent capacity is approaching to its maximum value and no more butyrate is removed in the extractor. Figure 5 also shows that the value of the final butyrate productivity increases asymptotically with solvent flowrate in the extractor, F_S. This result is expected since the amount of butyrate removed per unit time can be assumed proportional to the value of F_S for low values of the latter. Increasing F_S over a certain value is unproductive, since above this value there would be not much more butyrate available in the extractor's aqueous phase.

The predicted purity of the extract as a function of F_R and F_S is plotted in Figure 6. At low F_R values there is not adequate butyrate removal from the broth and, as a result, the pH in the extractor aqueous phase remains relatively low. But, since the ratio of the distribution coefficients of butyrate to acetate is an increasing function of pH (Figure 3), it is to be expected that the purity of the extract will increase with F_R. Indeed, this is the case as shown in Figure 6, where for a given value of F_S the extract purity increases with F_R. In the same plot, we also observe that the extract purity will decrease rapidly with increased solvent flowrate. This result reflects the well accepted "rule of thumb" in conventional

TABLE 3
Kinetic Constants for *B. methylotrophicum* as Determined from Batch Fermentation Data*, Through Non-Linear Optimization

μ_{max} = 0.187 h^{-1}	K_s = 4.18 mmol glucose/L
I_{ac} = 4.84 mmol acetate/L	I_{but} = 5.85 mmol butyrate/L
$Y_{x/s}$ = 0.2419 [cell OD_{660} units] / [mmol glucose/L]	
k_d = 0.041 h^{-1}	
a_a = 2.260 [mmol acetate/L] / [cell OD_{660} units]	
a_b = 0.340 [mmol butyrate/L] / [cell OD_{660} units]	
b_a = 0.000 [mmol acetate/L] / [cell OD_{660} units] / h	
b_b = 0.066 [mmol butyrate/L] / [cell OD_{660} units] / h	
$Y_{ac/s}$ = 2.0 mol acetate/mol glucose**	
$Y_{but/s}$ = 1.0 mol butyrate/mol glucose**	

*The initial substrate and cells concentration are 21.2 mmol glucose/L and 0.12 cell OD_{660} units, respectively.
**Determined from the biochemical pathway.

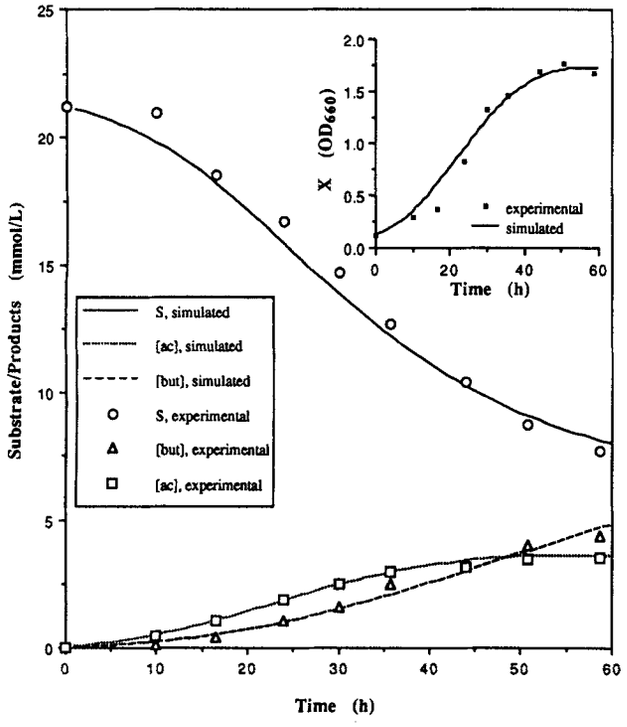

Figure 4 — Non-linear fitting of the batch fermentation data for *B. methylotrophicum* (Shen et al., 1988).

extraction processes that "several smaller extracts give higher purity than one very large extract".

In the rest of our simulation we used a recycle flowrate (F_R) equal to 0.6 fermenter volumes per hour, and a solvent flowrate (F_S) equal to 4.0 L/h. These values may be considered a good compromise between productivity and purity for the particular conditions of our system.

The buffer strength of the fermentation broth must also play an important role in the performance of the system, since the K_D values of the products are strong functions of pH (Equations (3) and (4)). This parameter is introduced into the complete model by altering the buffering salts concentrations of the medium in the pH-predicting equation that we used (Hatzinikolaou and Wang, 1991). Figure 7 shows the

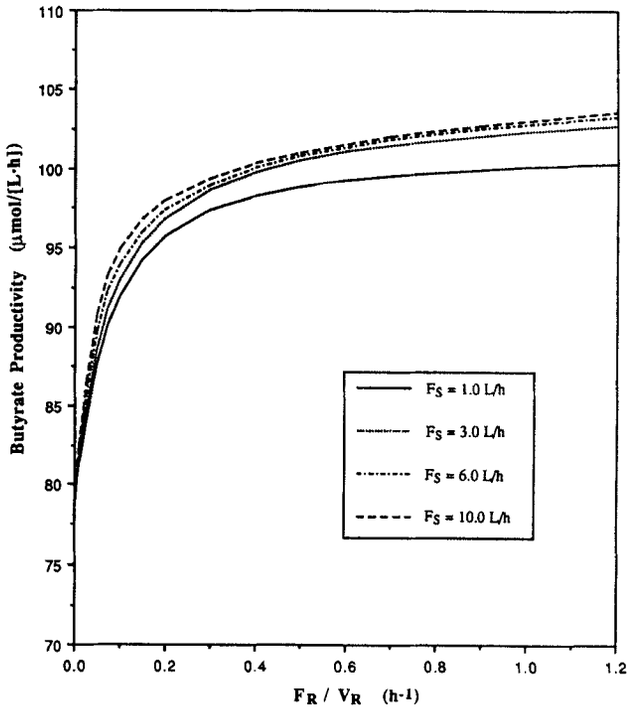

Figure 5 — Butyrate productivity as a function of recycle flowrate for different values of solvent flowrate.

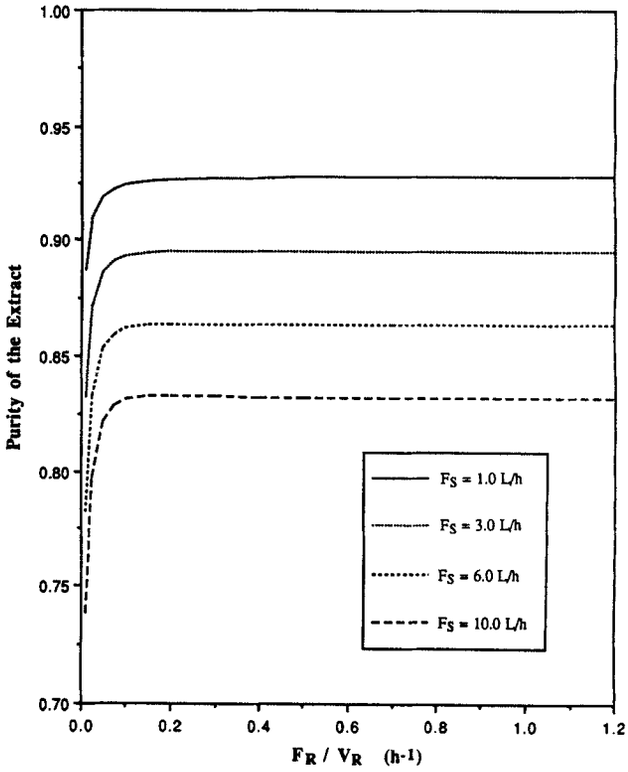

Figure 6 — Extract purity in butyrate as a function of recycle flowrate for different values of solvent flowrate.

relation between the two objective functions of the process and the buffer strength of the solution. As with F_S, the buffering capacity of the medium has conflicting effects on these two parameters. High buffering capacity of the broth means that the pH drop during the fermentation is slow. As a result, the pH of the medium remains high for longer periods of time, keeping the butyrate distribution coefficient low (inadequate product removal and, therefore, low

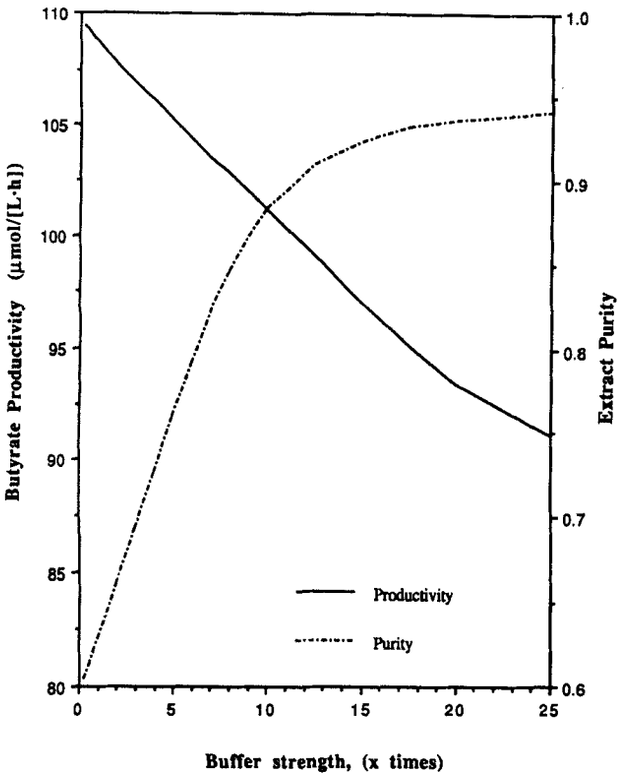

Figure 7 — Effect of medium buffering capacity on butyrate productivity and extract purity.

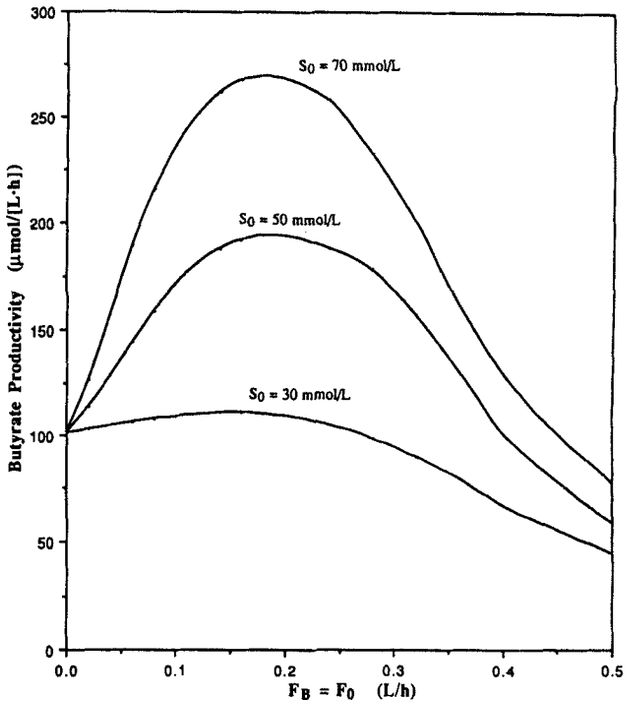

Figure 8 — Butyrate productivity vs bleed-feed flowrates at different inlet substrate concentrations.

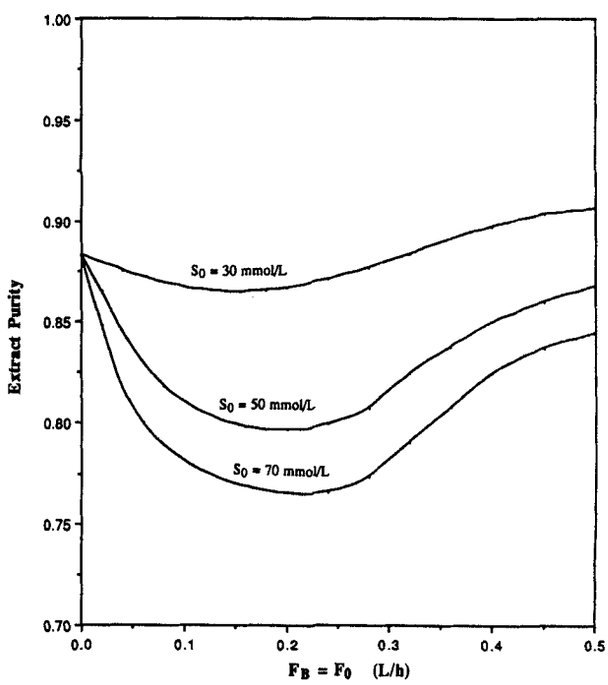

Figure 9 — Extract purity vs bleed-feed flowrates at different inlet substrate concentrations.

productivity) and the ratio of the K_D's high (high purity of the product).

The complete model now is applied in order to examine the effect of different operational modes on system performance. We compare the following four modes: batch fermentation, batch fermentation with recycle, fed-batch fermentation with recycle, and continuous fermentation with reycle. In order to choose the substrate feed concentration and the bleed and feed flowrates to use for the comparison among the different operational modes, we examined the effect of these parameters on the objective functions of the system (Equations 26 and 27) for the case of operation in a continuous mode. Figures 8 and 9 present the corresponding simulation plots. For a constant substrate concentration in the feed stream (S_0) there is an optimum value of the bleed and feed flowrates with respect to butyrate productivity. As the value of $F_B = F_0$ increases, removal of acids from the fermentation broth through the bleed stream reduces their inhibition effect on cell growth and more acids to be removed from the extractor are produced. The pH decreases, and the amount of butyrate extracted increases, while the purity of the extract decreases. Further increase in the value of $F_B = F_0$ results in a significant loss of butyrate through the bleed, dilution of the fermentation broth and increased pH values. Increasing the substrate concentration of the feed also results in asymptotically higher values of butyrate productivity since substrate also induces cell growth.

Based on the numerical results for the particular conditions of our model system plotted in Figures 8 and 9, in the fed-batch mode we selected a feed stream containing 50 mmol/L glucose and a feed volumetric flowrate F_0 = 0.15 L/h. Additionally, an equal bleed flowrate (F_B) was used in the continuous mode. Figure 10 shows the predicted pH of the aqueous phase in the fermenter for different operating modes. A qualitative review shows the benefits of online extraction through contact of the recycle stream with the solvent. The pH drop in the fermenter's aqueous phase is less steep for the extractive operation modes than for regular batch fermentation. This is very important since the microorganism can grow only in a limited pH range. As a result, operation time can be substantially lengthened, resulting in increased specific productivity (mmol butyrate/g cells). For the batch recycle mode (no feed or bleed stream) we observe that the pH profile reaches a minimum which

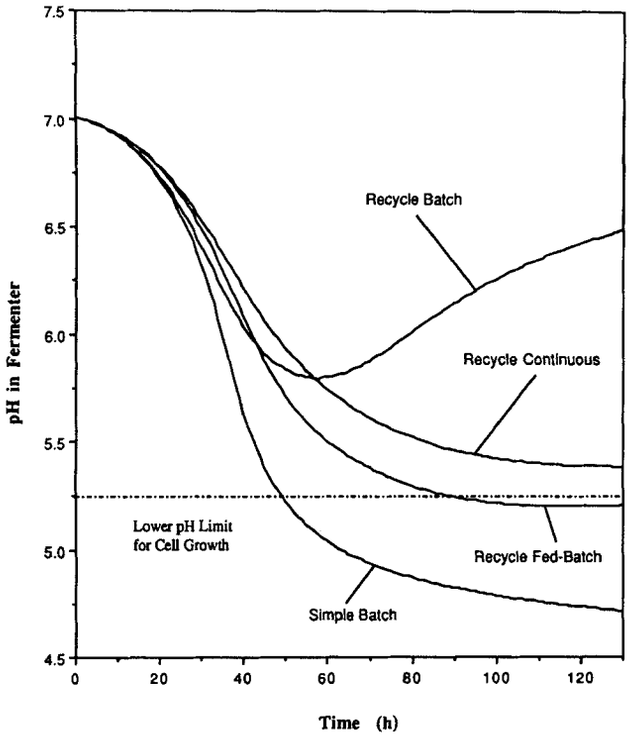

Figure 10 — pH profile in the fermenter at different operating modes.

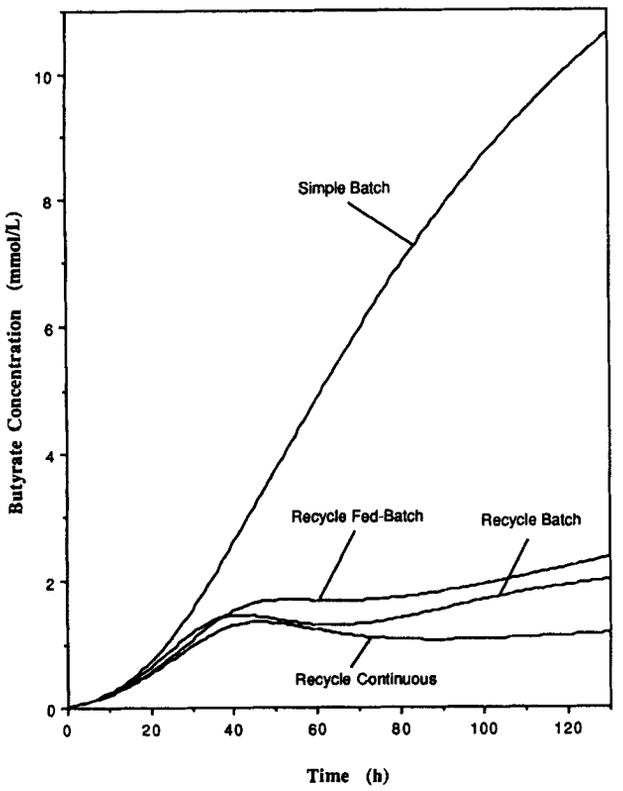

Figure 11 — Butyrate concentration profile in the fermenter for the different operating modes.

appears when the butyrate removal rate in the extractor becomes higher than the rate of its production by the cells. Subsequent increase of the pH is a disadvantage of the batch recycle operation since at high pH values the distribution coefficients of the products are low. The recycle continuous mode, as shown by Figure 10, could be considered as the most appropriate operational mode, since it gives relatively low pH values which do not limit cell growth.

TABLE 4
Butyrate Productivity and Extract Purity for the Different Operating Modes

Operating Mode	Butyrate Productivity μ mol/L · h	Purity of Extract %
Regular Batch	77.7	—
Batch recycle	105.5	91.0
Fed-batch recycle	158.9	86.4
Continuous recycle	166.5	79.7

Figure 11 shows the predicted butyrate concentration profile in the fermenter. As we would expect, butyrate concentration in the fermenter is much lower for the extractive modes. For the batch extractive fermentation, after an early maximum, the amount of butyrate in the fermenter decreases, since its production rate falls below its removal rate due to substrate exhaustion and cell death. For the fed-batch and continuous recycle modes, butyrate concentration in the fermenter follows a similar pattern. Figure 11 clearly depicts an additional advantage of the continuous operation mode. The existence of the bleed stream in this mode yields a relatively low concentration of acids in the fermenter. This result is very beneficial to the overall performance of the system since acids inhibit cell growth. In Figure 12, simulation results for the total butyrate produced (extracted + residual) are plotted as a function of time for the different operating modes. It is clear that the on-line extraction modes greatly increase the amount of butyrate produced compared to the regular batch fermentation. Among them, the continuous recycle and fed-batch recycle modes yield the highest butyrate production. The corresponding final productivities of butyrate for the four modes along with the purity of butyrate in the extract, are given in the Table 4. On-line extraction of the organic fermentation product is predicted to yield considerably higher productivity compared to the regular batch fermentation. In addition, on-line extraction provides a relatively effective gross separation of the two acids, yielding a butyrate purity in the extract well above 75%. The increase in productivity can be as high as 115% for the case of a continuous feed/bleed mode, although product purity is favored by the batch recycle mode.

Conclusions

In this work we examine the basic characteristics of on-line extractive fermentation of organic acids using the butyric acid fermentation as a model system. A model for predicting pH profiles during fermentation was combined with a biochemical kinetic model and the mass balance equations for continuous extraction. Using this complete on-line extraction model, a number of computer simulations were conducted in order to evaluate the effects of the various process parameters and operation modes on system performance.

Our results showed that on-line extractive fermentation of organic acids is very promising for increasing fermentation yield and productivity compared to regular batch fermentation. This result is mainly due to the removal of

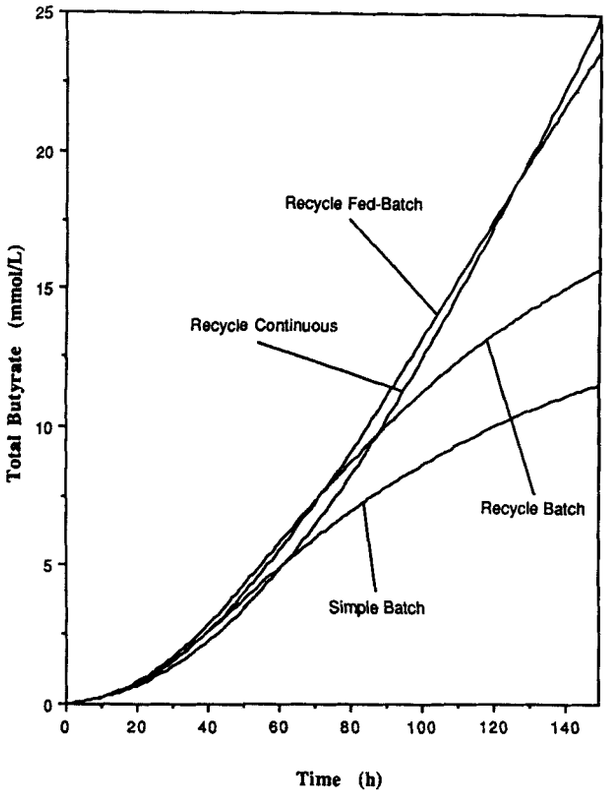

Figure 12 — Total butyrate produced per unit fermenter volume vs fermentation time, for the different operating modes.

acidic products which inhibit cell growth. In addition, when more than one acid is produced, extractive fermentation can simultaneously provide an effective separation of the more desirable product under conditions of favourable distribution coefficients. However, high volumetric productivity conflicts with purity of the extract. Thus, in order to run the system in the most effective manner, values of the process parameters will have to be determined through additional cost minimization equations. Complete operational models, like the one developed in this work, should play a key role for such work.

Acknowledgements

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Nomenclature

a_a = growth associated kinetic constant for acetate production, mmol acetate/g cells
a_b = growth associated kinetic constant for butyrate production, mmol butyrate/g cells
$[ac]$ = acetate concentration, mmol/L
$[ac]_E^A$ = acetate concentration in extractor aqueous phase, mmol/L
$[ac]_E^O$ = acetate concentration in extractor organic phase, mmol/L
$[ac]_0$ = acetate concentration in the feed, mmol/L
$[ac]_R$ = acetate concentration in the fermenter, mmol/L
A_{ext} = total amount of acetate extracted up to t_f, mmol
b_a = non-growth associated kinetic constant for acetate production, mmol acetate/g cells · h
b_b = non-growth associated kinetic constant for butyrate production, mmol butyrate/g cells · h
B_{ext} = total amount of butyrate extracted up to t_f, mmol
B_{tot} = total butyrate produced per fermenter volume, mmol/L
$[but]$ = butyrate concentration, mmol/L
$[but]_E^A$ = butyrate concentration in extractor aqueous phase, mmol/L
$[but]_E^O$ = butyrate concentration in extractor organic phase, mmol/L
$[but]_0$ = butyrate concentration in the feed, mmol/L
$[but]_R$ = butyrate concentration in fermenter, mmol/L
F_B = bleed line volumetric flowrate, L/h
F_0 = feed volumetric flowrate, L/h
F_R = recycle volumetric flowrate, L/h
F_S = solvent volumetric flowrate, L/h
I_{ac} = acetate inhibition constant on growth, mmol acetate/L
I_{but} = butyrate inhibition constant on growth, mmol butyrate/L
$K_{D\text{-}ac}$ = partition coefficient of acetate in solvent-water mixture
$K^*_{D\text{-}ac}$ = intrinsic partition coefficient of acetate, applied at low pH values
$K_{D\text{-}but}$ = partition coefficient of butyrate in solvent-water mixture
$K^*_{D\text{-}but}$ = intrinsic partition coefficient of butyrate, applied at low pH values
K_{ac} = acetate dissociation constant, mol/L
K_{but} = butyrate dissociation constant, mol/L
k_d = cell death rate constant, h^{-1}
K_s = inhibition constant on growth for the substrate, mmol substrate/L
P = extract purity, defined by Equation (27)
pK_a = $-\log_{10}$ [ionization constant of component a in water]
R_c = rate of cell growth, g cells/L · h
R_s = rate of substrate consumption, mmol substrate/L · h
R_{ac} = rate of acetate production, mmol acetate/L · h
R_{but} = rate of butyrate production, mmol butyrate/L · h
S = substrate concentration, mmol/L
S_E = substrate concentration in extractor (aqueous phase), mmol/L
S_R = substrate concentration in the fermenter, mmol/L
S_0 = substrate concentration in the feed, mmol/L
t = time, h
t_f = time required to reach the maximum rate of butyrate production, h
V = butyrate productivity (Equation 26), mmol/L · h
V_R = fermenter working volume, L
V_E = total extractor working volume, L
V_E^A = extractor aqueous phase volume, L
V_E^O = extractor organic phase volume, L
X = cell concentration, g/L
X_R = cell concentration in the fermenter, g/L
X_E = cell concentration in the extractor (aqueous phase), g/L
X_0 = cell concentration in feed, g/L
$Y_{ac/s}$ = substrate yield coefficient for acetate, mmol acetate/mmol substrate
$Y_{but/s}$ = substrate yield coefficient for butyrate, mmol butyrate/mmol substrate
$Y_{x/s}$ = substrate yield coefficient for biomass, g cells/mmol substrate

Greek

μ_{max} = maximum specific growth rate, h^{-1}

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