Communication to the Editor

Quantitative Evaluation of the pH Profile in Organic Acid Fermentations

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One of the most important considerations in designing an integrated process for the production of organic acids is the strong pH dependence of the process parameters. The release of the acidic products during the fermentation alters the pH of the broth and subsequently affects the kinetics of cell growth and product formation. In addition, some parameters (such as the distribution coefficient of the products during extraction, their solubility constants during precipitation, etc.) in the subsequent separation processes following fermentation are also strong functions of pH.

A pH profile predicting model for organic acids fermentation could be used in combination with specific separation process equations, in order to perform simulations concerning the feasibility of the whole process or the evaluation of the system's performance. In this work, such a general pH profile predicting model has been developed. The model requires knowledge of the fermentation medium composition and the kinetics of cell growth and product formation. The model has been verified for (but by no means restricted to) the case of butyric acid fermentation.

pH PROFILE MODEL DEVELOPMENT

In order to derive a model that accurately predicts the pH trajectory in a fermentation, it is essential to know the concentrations of all the pH affecting compounds in the bioreactor as a function of time. The term *pH affecting compound* includes all kinds of acids and bases (Lewis definition) as well as their salts. For the case of organic acids fermentation, the pH of the fermentation broth is mainly determined by the following two factors: (1) cell growth characteristics and (2) medium characteristics. The major difference between these factors is that the first is time dependent while the second is practically not. In the analysis that follows the contributions of both these factors will be examined.

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Cell Growth

The growth of the organic acid bacteria cells during fermentation can be described by the following general nonstoichiometric equation:

$$\text{Cells} + \text{substrate} + NH_4^+ \longrightarrow \text{more cells} + \text{acids} + H_2 + CO_2 \quad (1)$$

The substrates used in this kind of fermentation are carbohydrates (glucose). These compounds are characterized by very low dissociation constants in aqueous solutions, and as a result, they do not exhibit any significant acid/base properties. The cells in the fermentation broth may also affect the pH of the solution, since their membranes consist of a number of ionized compounds and since a number of extracellular ionized products (proteins and amino acids) may be released in the solution by them. At this point of the analysis, this effect is neglected on the assumption that cell concentration is usually low compared to the concentration of the other pH affecting compounds. Carbon dioxide is an acid gas and is produced during the fermentation. It may exist in the liquid phase in any of its four forms (CO_2, H_2CO_3, HCO_3^-, and CO_3^{2-}) and its dissociation could affect the pH of the broth. However, the pH at which organic acid fermentations take place is usually between pH 4.0 and 6.5. Considering the values of the dissociation and solubility constants for carbon dioxide below pH 6.0, the dissolved but undissociated CO_2 is the dominating species.[1] As a result, it is reasonable to neglect the effect of dissolved CO_2 on the pH of the culture. Finally, the only substantial pH affecting species from cell growth are the ammonium ion (NH_4^+) and the organic acids. Their concentrations are time dependent during the fermentation.

By solving numerically the equations that describe the particular fermentation kinetics, we obtain the concentrations of the organic acids produced and ammonium ions as a function of time. The dissociation of a monoprotic organic acid i in the aqueous fermentation

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broth is given as

$$OA_iH \longleftrightarrow OA_i^- + H^+ \quad (2)$$

The amount of H^+ ions released in the solution from OA_iH is equal to its dissociated concentration part x_i^t, $(x_i^t = [OA_i^-]^t)$.

According to the definition of the dissociation constant for the particular acid, the following equation must be valid for all times:

$$K_i = \frac{[OA_i^-]^t[H^+]^t}{[OA_iH]^t} = \frac{x_i^t[H^+]^t}{a_i^t - x_i^t}$$

or by rearrangement,

$$x_i^t = \frac{K_i a_i^t}{K_i + [H^+]^t} \quad (3)$$

where a_i^t is the total acid concentration $(a_i^t = [OA_iH]^t + [OA_i^-]^t)$ and K_i the corresponding dissociation constant. Note that the superscript t means a time dependent concentration. The effect of the dissociation of di- and triprotic organic acids in the H^+ concentration of the fermentation broth is treated in the Appendix. Similarly, for ammonium ion dissociation,

$$NH_4^+ \longleftrightarrow NH_3 + H^+ \quad (4)$$

the following equilibrium equation must also be valid:

$$x_{amm}^t = \frac{K_{amm} n^t}{K_{amm} + [H^+]^t} \quad (5)$$

where x_{amm}^t is the dissociated concentration part of the ammonium ion, n^t its total concentration, and K_{amm} the corresponding equilibrium constant.

Equations (3) and (5) determine the contribution of the cell growth time dependent concentrations (organic acids and ammonium ion) to the concentration of hydrogen ion in the fermentation broth. This contribution is equal to the sum of the undissociated concentration parts of the species involved and thus is equal to

$$[H^+]^t_{\text{cell growth}} = \sum_{i=1}^{q} x_i^t + x_{amm}^t \quad (6)$$

where q is the number of the different organic acids produced by the microorganism of interest.

Culture Medium

A culture medium of well-defined composition is considered here. This means that the initial concentrations of all the chemical species present are known. As mentioned earlier, characterization of a species in the fermentation broth as an acid or a base is based on the Lewis definition. Therefore, conjugate ions of weak acids (e.g., SO_4^{2-}) or weak bases (e.g., Ca^{2+}) occurring after the complete dissociation of a salt are considered as basic or acidic compounds, respectively. Their dissociation constants are $Kb_i = K_w/K$ and $Ka_i = K_w/K$, where K is the dissociation constant of the corresponding acid or base and K_w the dissociation constant of water. In the following analysis, we assume that the concentrations of the medium constituents remain constant with time or, in the worst case, that any change in these concentrations is negligible compared to the changes of the concentrations of the products and substrate.

Let $[AT]_i$ be the total acid concentration of the acid i in the broth. If xa_i is the dissociated concentration part of this acid, then again the well-known dissociation reaction occurs:

$$AH \rightleftharpoons A^- + H^+$$

According to the definition of the dissociation constant, we get

$$Ka_i = \frac{([A^c]_i + xa_i)[H^+]^t}{[AT]_i - xa_i}$$

or by rearranging,

$$xa_i = \frac{Ka_i[AT]_i - [A^c]_i[H^+]^t}{Ka_i + [H^+]^t} \quad (7)$$

where Ka_i is the dissociation constant of this acid, and $[A^c]_i$ is the concentration of the conjugate base of this acid that was added in the initial solution. Here $[A^c]_i$ is usually zero unless the pair AH–A^- is used as an acid–salt buffer system in the broth. Similarly, for the bases in the fermentation broth we get the following expression:

$$xb_i = \frac{Kb_i[BT]_i - [B^c]_i[OH^-]^t}{Kb_i + [OH^-]^t} \quad (8)$$

where $[BT]_i$ is the total added concentration of the i base, xb_i is the dissociated concentration part of it, $[B^c]_i$ is the added concentration of its conjugate acid in the initial solution, and Kb_i is its dissociation constant.

Final Model Formulation

Consider the dissociation of water molecules:

$$H_2O \rightleftharpoons H^+ + OH^-$$

From the definition of K_w, we have

$$K_w = [H^+]^t \cdot [OH^-]^t \quad (9)$$

Since the hydroxyl ion in the solution arises from the dissociation of all the bases including the water molecules, the hydroxyl ion concentration can be written as

$$[OH^-]^t = x + \sum_{i=1}^{m} xb_i \quad (10)$$

where x is the concentration of the hydrogen and hydroxyl ions occurring from the dissociation of water and m is the number of bases in the fermentation broth.

Substituting (9) into (10), we obtain

$$x = \frac{K_w}{[H^+]^t} - \sum_{i=1}^{m} xb_i \tag{11}$$

The xb_i's in Equation (11) are given by Equation (8) and depend on $[OH^-]^t$. But since the equilibrium implied by Equation (9) must always hold in the solution, $[OH^-]^t$ is equal to $K_w/[H^+]^t$. Thus x, as implied from Equation (11), is practically a function of $[H^+]^t$. The concentration of hydrogen ions in the solution at every time step is the sum of the contributions of all the acidic species in the fermentation broth: water ions, medium acids, and cell growth acids. Thus it is given by the following equation:

$$[H^+]^t = x + \sum_{i=1}^{n} xa_i + [H^+]^t_{\text{cell growth}}$$

and from Equations (6) and (11),

$$[H^+]^t = \frac{K_w}{[H^+]^t} - \sum_{i=1}^{m} xb_i + \sum_{i=1}^{n} xa_i + \sum_{i=1}^{q} x_i^t + x^t_{\text{amm}} \tag{12}$$

where n is the number of acidic species [other than the fermentation products (organic acids) and the ammonium ions] in the solution. Since the xa_i's, xb_i's, x_i^t's, and x^t_{amm} are functions of $[H^+]^t$, Equation (12) is a nonlinear function of $[H^+]^t$, which can be solved numerically to obtain the pH of the solution at every time step (new values of a_i^t's and n^t).

TESTING THE pH PROFILE MODEL

As indicated in the previous analysis, it is necessary to know the exact composition of the culture medium in order to predict the pH evolution with time during the batch fermentation. The above constraint reduces the applicability of our model only to well-defined culture media. Molasses, starch, and whey, for example, are substrates of undefined composition and consequently cannot be used in verifying our model. On the other hand, although organic acids fermentation is a well-studied subject, batch fermentation data without pH control are quite limited. In the following analysis, we tested the predicting ability of the pH model using two data sources found in the literature that satisfy the above stated constraints. Both cases concern the batch fermentation of *Clostridium acetobutylicum*. This bacterium can anaerobically ferment a variety of sugars to produce a number of organic solvents (e.g., butanol, acetone, and ethanol) and carboxylic acids (acetate, butyrate, and lactate). Batch fermentation of these bacteria exhibits a two-phase scheme. The first phase consists of a rapid bacterial growth associated with active acetate, butyrate, CO_2, and H_2 production (acidogenic phase). In the second phase, the specific growth rate decreases, and solvent formation takes place (solventogenic phase), accompanied by partial uptake of the acids formed during the first growth phase.[2]

In case I,[3] the authors performed a series of pH controlled batch fermentations of *C. acetobutylicum* [NCIB 8052 (ATCC 824)] in order to study the production of solvents by this bacterium at various pH values. Along with these experiments, they ran an uncontrolled pH batch fermentation. In case II,[4] the author, working on the production of solvents by *C. acetobutylicum* (ATCC 824), performed some batch fermentation experiments in Hungate tubes. The uncontrolled pH experiment data will be used in verifying the model. Since we are mainly interested in testing the predictability of our pH model during a general organic acid fermentation (no production of solvents), we are going to use the data concerning only the acidogenic phase of the batch fermentation. This fact considerably simplifies the biochemical kinetic model to be used. During the initial stage (acidogenic phase) of the batch fermentation of *C. acetobutylicum* the two major metabolic products released are acetic and butyric acids. We may assume that substrate is still in excess, while the concentrations of the products have not yet reached any significant inhibition levels. Also, the cell's concentration is quite low to account for any significant death rate, and no solvent production has taken place yet. Under these assumptions, cell growth is given by the following exponential growth equation:

$$R_c = \frac{dX}{dt} = \mu_{\max} X \tag{13}$$

where R_c is the cell's growth rate, X, the cell's concentration (g/L) and $\mu_{\max}$, the cell's specific growth rate (h^{-1}). For the product formation kinetics we can use a mixed growth model[5] of the Luedeking–Piret type:

$$\frac{d[\text{ac}]}{dt} = a_a R_c + b_a X \tag{14}$$

$$\frac{d[\text{but}]}{dt} = a_b R_c + b_b X \tag{15}$$

where [ac] is in grams total acetate per liter, [but] is in grams total butyrate per liter, $a_{a,b}$ is in grams acid per gram of cells, and $b_{a,b}$ is in grams acid per gram of cells per hour.

Equations (13)–(15) are easily integrated in order to give the cell and product concentrations as a function of time:

$$X = X_0 \exp(\mu_{\max} t) \tag{16}$$

$$[ac] - [ac]_0 = \frac{a^* X_0}{\mu_{\max}} [\exp(\mu_{\max} t) - 1] \tag{17}$$

$$[\text{but}] - [\text{but}]_0 = \frac{b^* X_0}{\mu_{\max}} [\exp(\mu_{\max} t) - 1] \tag{18}$$

where X_0 is the initial bacterial concentration (g cells/L), $[ac]_0$ and $[but]_0$ are the initial acetate and butyrate concentrations (g acid/L), and a^* and b^* are lumped constants (g acids/g cells h) which are functions of $a_{a,b}$, $b_{a,b}$, and $\mu_{\max}$. The constants in equations (16)–(18) can

be easily determined from the available experimental data using linear regression.

At this point, with all the constants of the fermentation model determined from the experimental data, the pH model can be used in order to predict the pH of the solution during the acidogenic phase. In Table I we list the concentrations of the compounds that significantly affect the pH in the culture medium.

Case I

According to Equation (12) and the species in Table I, the resulting pH profile predicting equation for case I must be the following:

$$[H^+]^f = \frac{K_w}{[H^+]^f} - \frac{Kb_1[SO_4^{2-}]}{Kb_1 + K_w/[H^+]^f} + \frac{Ka_1[H_2PO_4^-] - [HPO_4^{2-}][H^+]^f}{Ka_1 + [H^+]^f} + \frac{Ka_2[Mg^{2+}]}{Ka_2 + [H^+]^f} + \frac{K_{ac}[ac]^f}{K_{ac} + [H^+]^f} + \frac{K_{but}[but]^f}{K_{but} + [H^+]^f} + \frac{K_{amm}[NH_4^+]}{K_{amm} + [H^+]^f} \quad (19)$$

Equation (19) is the final nonlinear equation of the pH model that has to be solved at each time step (since $[ac]^f$ and $[but]^f$ change) in order to give the pH of the solution. The values of the dissociation constants in Equation (19) correspond to the particular fermentation temperature.[6] The predicted pH profile along with the values of the experimental points are given in Figure 1. In the same figure we include the predictions of our pH predicting equation as determined from the real time experimental values of acetate and butyrate. It is obvious that the error introduced by the linear regression process is negligible.

We observe that the model yields quite accurate predictions considering the complexity of the pH nature. The maximum error is only ca. 4% (including the linear regression one). The small deviations from the experimental values are inevitable since we are dealing with such a sensitive parameter as the pH, where even its experimental measurement is not error free. In the particular example, the error sources may be the following: (a) the existence of living cells in the fermentation broth

Table I. pH affecting compounds in the culture medium for cases I and II (g/L distilled water).

Substance	Case I	Case II
KH_2PO_4	3.35	0.75
K_2HPO_4	3.35	0.75
NH_4Cl	3.00	—
$MgSO_4 \cdot 7H_2O$	0.40	—
$MgSO_4$	—	0.20
Asn · H_2O	—	0.50
Cys	—	0.50
Yeast extract	—	5.00

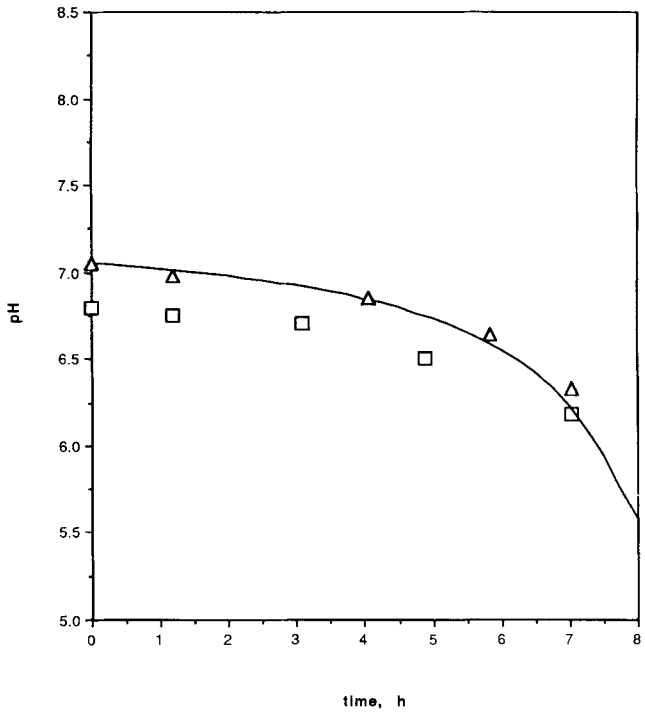

Figure 1. Predicted pH profile (——) and predicted values from real time acid concentrations (□) vs. experimental data (△) for case I. The values of the kinetic constants were determined as μ_{max} = 0.287 h^{-1}, X_0 = 0.036 g/L, a^* = 0.602 g acetate/g cells/h, and b^* = 0.773 g butyrate/g cells/h.

since, in addition to the acids, a number of extracellular ionized polypeptides or proteins may be released into the solution, and (b) the assumption that the concentrations of all the species (except acetate and butyrate) are assumed to be constant. This might be true for some ions such as SO_4^{2-}, but it is certainly not true for NH_4^+ and phosphate ions. In fact, our model in Equation (12) is constructed for a variable total NH_4^+ concentration, and as a result, in the fermentation kinetic model we have to use an additional equation describing the uptake of NH_4^+ by the microorganism. Unfortunately, in the studied case no measurements of the NH_4^+ concentration during the fermentation were taken. Phosphate consumption may also introduce an error. In fact, many *C. acetobutylicum* cultures have been reported to be phosphate limited. The knowledge of the phosphate consumption kinetics would certainly improve the predictability of the model.

However, the relatively good agreement of the simulation results with the experimental data indicates that the effect of the above error sources is not so important, and the model, as it is, can predict the pH profile during the organic acids fermentation with reasonably good accuracy. This result is probably due to the fact that the dissociation constants of the two acids produced are 10^3–10^4 times greater than those of NH_4^+ and phosphate ions. Thus, we may conclude that the dissociation of the organic acids produced is the major factor that determines the pH profile in the fermentation broth.

Case II

As we see from Table I in this case a more complex medium was used. A problem that arises in this study is that a relatively high concentration of yeast extract was used in the culture medium and could not be neglected. Since the composition of yeast extract is not defined, we ran some independent titration experiments to determine its influence on the pH of the solution. The nonlinear regression calculations show that a solution of 5 g/L of yeast extract can be considered as a buffer of a weak acid of initial concentration $[YH] = 7.16 \times 10^{-2}$ in equilibrium with its salt anion of initial concentration $[Y^-] = 2.14 \times 10^{-3}$. The equilibrium constant of this hypothetical acid was determined as 1.74×10^{-8}. Also in this medium no ammonium ion source has been used. By taking the above facts into account and according to Equation (12), the pH profile predicting equation for case II becomes

$$[H^+]^f = \frac{K_w}{[H^+]^f} - \frac{Kb_1[SO_4^{2-}]}{Kb_1 + K_w/[H^+]^f} - \frac{Kb_2[Asn]}{Kb_2 + K_w/[H^+]^f} - \frac{Kb_3[Cys]}{Kb_3 + K_w/[H^+]^f} + \frac{Ka_1[H_2PO_4^-] - [HPO_4^{2-}][H^+]^f}{Ka_1 + [H^+]^f} + \frac{Ka_2[Mg^{2+}]}{Ka_2 + [H^+]^f} + \frac{Ka_3[/Asn]}{Ka_3 + [H^+]^f} + \frac{Ka_4[Cys]}{Ka_4 + [H^+]^f} + \frac{Ka_5[Cys]}{Ka_5 + [H^+]^f} + \frac{Ka_6[YH] - [Y^-][H^+]^f}{Ka_6 + [H^+]^f} + \frac{K_{ac}[ac]^f}{K_{ac} + [H^+]^f} + \frac{K_{but}[but]^f}{K_{but} + [H^+]^f} \quad (20)$$

Note that in this medium that amino acids contribute to the pH through both their acidic and basic groups. Cysteine in particular has two acidic groups each treated as a separate acid. Also no ammonium ion source was used. Equation (20) is solved along with the kinetic model to give the pH profile of the case II batch fermentation. This profile, along with the measured pH values and the real time acid concentration predictions, are given in Figure 2. Again, our model predicts the pH profile quite accurately (maximum error ca. 3%). This is quite important, since the fermentation broth of this example is more complex than the previous one. The relatively better predictions in case II compared with the predictions in case I could be the result of the absence of ammonium ion in the fermentation broth of case II, a fact that effaces the error of NH_4^+ consumption kinetics.

CONCLUSIONS

In this work, a general predicting model for the pH profile in the fermentation broth of organic acid fermentation has been developed. The model has been successfully tested against two different sets of experimental data for the acidogenic phase of the batch fermentation of *C. acetobutylicum*. Our results have shown that it is possible to predict (with a reasonable degree of accuracy) the pH profile during a bacterial fermentation provided that the composition of the fermentation broth and the kinetic parameters of the cell growth are known. The accuracy in the predictions of our model is expected to reach high levels as the kinetics of the fermentation are more complete. Such a model could be used in providing useful information on the effect of the various pH affecting parameters in the fermentation kinetics or in performing simulations on integrated fermentation–separation systems where pH dependent factors are important in the overall system's performance and productivity. In fact, the use of this model has been proved essential during simulations concerning the on-line extractive fermentation of organic acids performed in our laboratory. This work will be published in the near future.

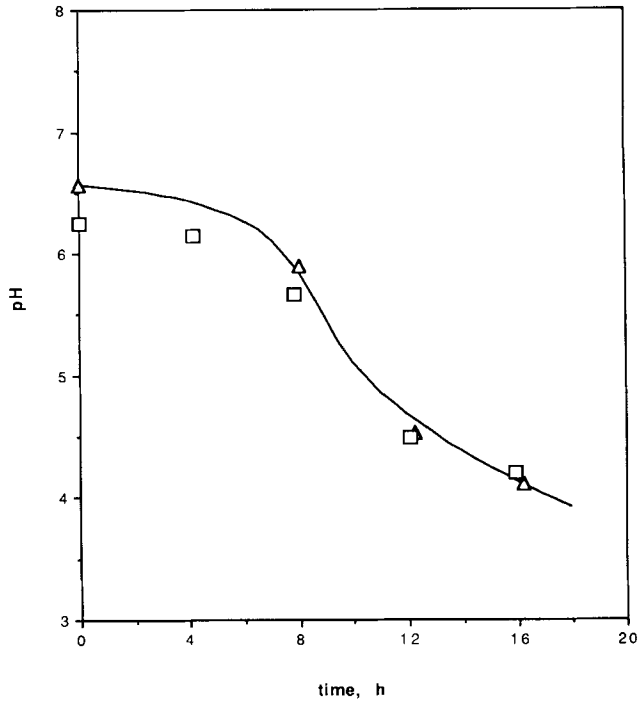

Figure 2. Predicted pH profile (——) and predicted values from real time acids concentrations (□) vs. experimental data (△) for case II. The values of the kinetic constants were determined as $\mu_{max} = 0.220\ h^{-1}$, $X_0 = 0.018$ g/L, $a^* = 0.468$ g acetate/g cells/h, and $b^* = 0.543$ g butyrate/g cells/h.

APPENDIX

If one of the fermentation organic acids is diprotic, the following dissociations take place upon its production.

$OA_iH_2 \rightleftharpoons OA_iH^- + H^+$ with dissociation constant K_i^1

$OA_iH^- \rightleftharpoons OA_i^{2-} + H^+$ with dissociation constant K_i^2

The equilibrium for the above set of reactions is given by the following equations:

$$K_i^1 = \frac{[OA_iH^-]^f[H^+]^f}{[OA_iH_2]^f} \quad (I)$$

and

$$K_i^2 = \frac{[OA_i^{2-}]^f[H^+]^f}{[OA_iH^-]^f} \quad (II)$$

The contribution of the H^+ concentration of the broth from this acid is equal to the sum of the dissociated concentration parts of OA_iH_2 and OA_iH^-. Thus:

$$x_i^f = [OA_iH^-]^f + [OA_i^{2-}]^f \quad (III)$$

Substitution of (I) and (II) into (III) yields:

$$x_i^f = \frac{K_i^1[OA_iH_2]^f}{[H^+]^f} + \frac{K_i^2[OA_iH^-]^f}{[H^+]^f}$$

$$= \frac{K_i^1[OA_iH_2]^f}{[H^+]^f} + \frac{K_i^1K_i^2[OA_iH_2]^f}{\{[H^+]^f\}^2}$$

$$\longrightarrow x_i^f = [OA_iH_2]^f\left[\frac{K_i^1}{[H^+]^f} + \frac{K_i^1K_i^2}{\{[H^+]^f\}^2}\right] \quad (IV)$$

If a_i^f is the total acid produced up to time t, then the following equation must hold at all times:

$$a_i^f = [OA_iH_2]^f + [OA_iH^-]^f + [OA_i^{2-}]^f$$

which according to Equation (III) yields:

$$a_i^f = [OA_iH_2]^f + x_i^f \quad (V)$$

Substitution of $[OA_iH_2]^f$ from (V) into (IV) gives the contribution of the dissociation of the diprotic acid OA_iH_2 in the H^+ concentration of the fermentation broth, x_i^f:

$$x_i^f = \frac{a_i^f[K_i^1[H^+]^f + K_i^1K_i^2]}{\{[H^+]^f\}^2 + K_i^1[H^+]^f + K_i^1K_i^2} \quad (VI)$$

In a completely analogous manner the contribution of the dissociation of a triprotic organic acid produced (OA_iH_3) in the H^+ concentration of the fermentation broth is given by the following equation:

$$x_i^f = \frac{a_i^f[K_i^1\{[H^+]^f\}^2 + K_i^1K_i^2[H^+]^f + K_i^1K_i^2K_i^3]}{\{[H^+]^f\}^3 + K_i^1\{[H^+]^f\}^2 + K_i^1K_i^2[H^+]^f + K_i^1K_i^2K_i^3} \quad (VII)$$

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