OPTIMIZATION OF BIOTECHNOLOGICAL PROCESSES. THE ACETIC ACID FERMENTATION. PART I: THE PROPOSED MODEL

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Abstract

Vinegar is a food product of increasing significance by virtue of its widely variable origin and uses (particularly as a condiment or food preservative). The gastronomic value of vinegar has been appreciated for thousands of years. The growing social and economic significance of these products has fostered research into the most salient aspects of their production processes. The widespread use of submerged cultures in such processes has aroused an obvious interest in their modelling with a view to facilitating their design, control and optimization. Also, the availability of increasingly powerful utility and dedicated software tools has enabled a much rigorous approach to devising and application of more complex and accurate models for these purposes. This paper (Part I) reviews previous attempts at modelling acetic acid fermentation and proposes a new mathematical model for the process based on extensive experimental testing. The model introduces new equations and considers cell lysis during the process. Part II is devoted to study the key subject of parameter estimation and finally Part III deals with the optimization task. Though the wine vinegar process is being considered, many of the studied issues could be applied to other fermentations.

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1. Introduction

Modelling acetic acid fermentation has aroused much interest for decades in response to the growing research into the process. A number of kinetic equations have been reported to describe experimental evidence on the influence of diverse variables of the process. One such equation [1], based on the Monod model [2], assumes the cell growth rate to be determined by oxygen availability in the medium. Previously, some authors had found oxygen to influence the fermentation process. Thus, Czuba [3] concluded that oxygen was the limiting substrate when its uptake fell below 10.5 mg $O_2 \cdot mg^{-1}$ biomass$\cdot h^{-1}$. Also, Oosterhuis *et al.* [4] found systems containing the gas at levels below 10% of its saturation concentration to be oxygen-deficient. Mori *et al.* [5] developed an expression very similar to one previously reported by Cho and Wang [1] except that the growth rate was a function of the oxygen partial pressure. In general, the previous models failed to consider the influence of the other substrate (ethanol) and the product (acetic acid).

Later on, however, Nanba *et al.* [6] focused on the analysis of the synergistic effect of the main substrate (ethanol) and product (acetic acid) on *Acetobacter* growth during acetic fermentation. By using low cell concentrations (15 $mg \cdot L^{-1}$) in order to ensure a negligible inhibitory effect, they examined the influence of ethanol on cell growth and developed an expression for the specific growth rate, based on a non-competitive inhibitory effect.

According to Nanba, acetic acid concentrations below 10 $g \cdot L^{-1}$ resulted in significantly increased rate growths (particularly at low ethanol concentrations). Above 20 $g \cdot L^{-1}$ acetic acid, however, growth was severely restricted —and virtually completely inhibited at an acetic concentration in the region of 50 $g \cdot L^{-1}$, whichever the amount of ethanol present.

The Nanba model was the first to consider the activating–inhibitory effect of acetic acid, and also its relationship to ethanol.

In subsequent experiments, Bar *et al.* [7] examined batch acetic fermentations at initial ethanol concentrations of 36.5 $g \cdot L^{-1}$ and found *Acetobacter aceti* to exhibit an abnormal behaviour: the product formation rate increased with decrease in the microbial growth rate. Ethanol evaporation losses during the tests were minimized by using a condenser to collect evolved gases at 4 ºC and with an aeration of 0.25 $mL \cdot min^{-1}$ in a 1.5 L reactor. This aeration rate resulted in an oxygen deficiency that was overlooked. Also, one of the main empirical conclusions drawn was that the ratio of ethanol uptake to acetic acid production was stoichiometric. Bar developed an exponential expression for the specific growth rate, μ_c, which decreased from a maximum level at the beginning of fermentation to near zero at the end.

Also, they found the product formation rate to peak at a relatively low acetic acid concentration, 12.5 $g \cdot L^{-1}$, which facilitated cell activity. The gradual increase in such a concentration during fermentation inhibited microbial growth —or even in substantial cell death above moderate concentrations (45 to 60 $g \cdot L^{-1}$).

In 1989, Park and co-workers published the first paper in a series describing the behaviour of *Acetobacter aceti* under diverse fermentation conditions and developed a kinetic model based on their findings. They used submerged cultures in a synthetic medium in combination with various operational modes and a filtering module to

recycle the biomass. The primary aim was always to maximize the acetification rate. In one study [8], they examined the effects of dissolved oxygen and acetic acid, and found microbial respiration to be optimal at a dissolved oxygen concentration of 3–7 $mg \cdot L^{-1}$ in the absence of acid and its presence to lower it to 1–2 $mg \cdot L^{-1}$. In a subsequent study [9], they examined the behaviour of bacteria in a 600 mL reactor containing a controlled dissolved oxygen concentration of 1–3 $mg \cdot L^{-1}$; they obtained high acetification rates (up to 120 $g \cdot L^{-1} \cdot h^{-1}$) with an output acetic concentration of 40 $g \cdot L^{-1}$ and a yield factor (acetic acid-to-ethanol ratio) of 1.28. In similar, subsequent work, they focused on continuous operation of the reactor [10]. Finally, in the last paper of their series [11], they reported a semi-empirical model for acetic acid fermentation.

The model is based on a scheme by Sinclair and Topiwala [12], but assumes bacteria to evolve during fermentation, and biomass to exist in a viable form capable of growing and consuming substrate and also in a non-viable form unable to grow but also consuming some substrate for maintenance.

This model is subject to two major shortcomings, namely:

(a) The consideration of non-viable biomass, which cannot grow but requires substantial energy is not properly justified (no measures of this kind of biomass are supplied).

(b) It can provide abnormal values for some specific rates under seemingly "normal" conditions. For example, it gives negative (*i.e.*, spurious) specific rates at typical initial acetic acid concentrations (about 10 $g \cdot L^{-1}$).

In subsequent works [13,14], these authors found new ways to raise the acetification yield without altering their model.

Ito *et al.* [15] substantially modified the original model of Park. Thus, they used Nanbas's expression rather than Park's for the specific growth rate in order to consider

the influence of ethanol. Also, they used the estimated parameter values previously employed by Nanba. One other major difference was that they used an exponential expression for the product to associate the specific rate of transformation of viable cells into non-viable cells, thereby excluding the possibility of obtaining negative values. Unlike the Park model, the specific rate of transformation of non-viable cells into dead cells was constant rather than exponentially variable.

Although they circumvented some of the shortcomings in the Park proposal, their model still failed to accurately account for some aspects such as the behaviour of non-viable cells.

Romero *et al.* [16] developed a model that attached specific physical significance to the parameters in the Nanba model. The new model was based on the experimental results obtained with a submerged culture of *Acetobacter aceti* in a wine medium as used under batch operation at the industrial and laboratory level, using open or closed gas recycling systems to avoid ethanol evaporation losses.

The microbial growth was a function of the concentrations of ethanol, acetic acid and oxygen, expressions considering activation-inhibition were proposed.

The model also used a combined influence of the substrate and product on cell death [17].

For the ethanol uptake a kinetic equation is proposed assuming that ethanol is only used to supply the amount of energy needed for biomass growth.

Maybe, the main shortcomings of the model are: on one hand, it cannot explain the total cell concentration since no lysis has been taken into account and, on the other hand, the kinetic equation for cell death considers the complete absence of oxygen in the medium, that it is not, normally, a real situation.

Kruppa and Vortmeyer [18] developed an empirical model using equations for the rates of cell growth, ethanol uptake and acetic acid production only depending on the acetic acid and the total cell concentrations. This model failed to distinguish between viable and non-viable cells; also, it calculated its parameter values from data for a continuous fermenter operating at 30 ºC under no oxygen supply restrictions.

Finally, González-Saiz *et al.* [19] reported a simplified model based on data from industrial fermentations. It considered viable and death biomass, disregarding non-viable biomass potentially consuming some substrate under the assumption that no industrial evidence of its presence had so far been provided. It does not consider growth restrictions arising from dissolved oxygen in the medium as it assumed that this could not be a limiting substrate since the typical aeration conditions used industrially were adequate to meet microbial oxygen demands. Also, it does not take into account potential inhibition by acetic acid on the grounds that in the range of the studied conditions this influence was not observed. In fact, it only considered growth restrictions due to an ethanol deficiency and assumes the specific rate of cell death to be constant throughout the process. As a result, the equation for cell growth assumed ethanol to be the sole limiting factor.

So, taking into account the shortcomings of the previously mentioned models and with the main objective of making a new model proposal which could overcome those limitations, an extensive experimental research aimed at identifying the optimum industrial operating conditions as regards the fermentation rate, revealed some variables to behave in a way or exert some effects that cannot be accurately described as a whole by any of the above-described models. Based on the raw materials and operating modes used, the influential factors ignored, and various other factors, we believed it of interest to develop a model exploiting some advantages of existing ones, but additionally

considering a previously unaddressed factor: cell lysis. In this way, we established new kinetic equations that fit experimental evidence more accurately than their previous counterparts.

2. Experimental results

A variety of tests aimed at assessing the influence of the major variables in the acetification process under near-industrial conditions were conducted within the framework of a more broad research.

A Frings 8 L fermentation tank was used. The operational procedure was a semicontinuous one, consisting on the depletion of ethanol in the medium to a concentration of 3.9, 15.5 or 27.1 $g \cdot L^{-1}$ at a constant temperature of 31 ºC and an also constant air flow rate of 7.5 L $air \cdot h^{-1} \cdot L^{-1}$ medium. Once the desired ethanol concentration was reached, a 25, 50 or 75 % of the tank contents were unloaded. After that, the tank was slowly loaded (feed rate of 0.035 $L \cdot min^{-1}$) to the final working volume (8 L) was completed; a Montilla-Moriles white wine (vinegar produced from which has recently obtained the mark of protected origin designation) containing 93.0 g $ethanol \cdot L^{-1}$ was used.

Acidity was determined by acid–base titration of samples periodically withdrawn from the fermentation medium and ethanol using on-line procedure involving Alkosens® probe from Heinrich Frings (Bonn, Germany). Also, total and viable cell counts were obtained by using the method of Baena *et al.* [20].

The bioreactor was fully equipped to operate in an automated mode. Loading, unloading, control and monitoring operations were performed unattended via a previously programmed computer.

In order to maximize the information extracted from each test and identify potential uncertainties, the tests were replicated at least 8 times under each set of operating conditions.

The variable unload tests involved, as said before, withdrawing 75, 50 or 25 % of the total working volume (tests A1, A2 and A3, respectively), *i.e.* leaving a residual volume of 2, 4 or 6 L, respectively, as inoculum for the next cycle. The ethanol concentration at unload was 15.5 $g \cdot L^{-1}$ in all cases.

On the other hand, the variable ethanol concentration tests were conducted with 27.1, 15.5 and 3.9 g ethanol$\cdot L^{-1}$ (tests B1, B2 and B3, respectively). The unloaded volume was 50 % in all cases. As can be seen, test B2 coincided with A2.

The most salient results of the tests are summarized in Table 1, where r_{ave} is the average fermentation rate as determined with a procedure described elsewhere [21], P the acetic acid production, t_{load} the duration of the loading stage, t_{cycle} that of the fermentation cycle, E_{load} the ethanol concentration at the end of the loading stage, HAc_{cycle} the acetic acid concentration at the end of the fermentation cycle and HAc_{mean} the mean acidity through out the cycle.

	r_{ave} $\left(\frac{g \text{ acetic acid}}{\text{L medium}\cdot h}\right)$	P (g acetic acid$\cdot h^{-1}$)	t_{load} (h)	t_{cycle} (h)	E_{load} $g \cdot L^{-1}$	HAc_{cycle} $g \cdot L^{-1}$	HAc_{mean} $g \cdot L^{-1}$
A1	2.1±0.1	15.1±0.5	3.3±0.1	37.5±1.1	69.0±0.8	95.0±1.0	56.0±20.0
A2 (B2)	2.3±0.1	17.1±0.5	1.8±0.1	23±0.6	48.8±0.8	98.0±1.0	73.0±13.0
A3	2.2±0.2	17.3±0.4	0.9±0.1	11.2±0.2	31.0±0.8	97.0±1.0	86.0±6.0
B1	2.2±0.2	16.3±0.4	2.0±0.1	19.2±0.4	55.0±0.8	78.0±5.0	62.0±11.0
B3	2.2±0.7	14.7±0.3	2.1±0.1	30.0±0.3	45.7±0.8	111.0±1.0	82.0±15.0

Table 1: Selected experimental results of the fermentation tests

The total and viable cell counts obtained allowed the average proportion of viable cells at any time during sampling to be calculated. Figures 1 and 2 show the proportions obtained in tests A1–A3 and B1–B3, respectively.

< Figure 1 >

< Figure 2 >

Finally, by way of example, Figure 3 shows the variation of the total concentration of cells during test B3.

< Figure 3 >

3. Discussion

As can be seen from the results of tests A1–A3, the maximum ethanol concentration obtained at the end of the loading stage varied markedly: from 31 $g \cdot L^{-1}$ in A3 to 69 $g \cdot L^{-1}$ in A1. Also, as can be seen from Figure 1, the proportion of viable cells decreased appreciably in A1 and A2. The reduction seemingly coincided with the loading period, which involved rapid changes in ethanol concentration and acidity. Such changes were especially marked in test A1, where the ethanol concentration rose from 15.5 $g \cdot L^{-1}$ at the start to the above-mentioned maximum level: 69 $g \cdot L^{-1}$. By contrast, the ethanol concentration in test A3 rose from 15.5 $g \cdot L^{-1}$ to only 31 $g \cdot L^{-1}$. The foregoing suggests that the ethanol concentration of the medium has some influence on cell viability. Thus, ethanol levels around and above 48.0 $g \cdot L^{-1}$ seemingly reduced the proportion of viable cells as well as influence the global bacterial activity and, hence, on the global behaviour of the process.

If the acetic acid production is compared for tests A1-A3, it can be concluded that was lower in test A1 than in the others, which might be consistent with the above-described reduction in cell viability.

This body of tests clearly exposed the influence of ethanol on cell viability. The decrease in the proportion of viable cells may have resulted both from inhibition of cell growth and from a direct action on viable cells leading to their death. Obviously, both phenomena might have occurred simultaneously, thereby strengthening the reduction in viable cells. All this suggests the need to expand the previous kinetic equations with appropriate terms in order to consider the influence of these variables. Additionally, regarding the potential influence of the acetic acid concentration on the cell activity, it can be stated that, although the average acidity during the fermentation cycle was higher in test A3 than in A1, this seemingly has not a negative influence on acetic acid production. If one considers the well-known inhibitory effect of acetic acid on acetic bacteria, then it seems obvious that no such effect was apparent from the results of this test series.

The primary difference between tests B1 to B3 was the ethanol concentration at unload, which was 27.1 $g \cdot L^{-1}$ in B1, 15.5 $g \cdot L^{-1}$ in B2 and 3.9 $g \cdot L^{-1}$ in B3. The unloaded volume fraction, however, was identical in all cases: 50 %.

Similarly to tests A1–A3, Figure 2 shows the variation of the average proportion of viable cell in each cycle. As can be seen, in test B1, a similar decrease to that in tests A1 and A2 is found (at final of loading stage); the reduction in viable cells, however, was smaller than the greatest observed in the previous tests (A1), which is seemingly consistent with the fact that the highest ethanol concentration, 55 $g \cdot L^{-1}$, was also lower than that reached in A1. The obtained results for B3 will be discussed later.

Comparing the acetic acid production obtained in tests B1–B3, the production values for tests B1 and B2 were similar to each other and slightly higher than those for B3.

Therefore, although this test series exposed a slight reduction in the proportion of viable cells in B1 and B2, the outcome was apparently governed by other variables such as the

low concentration of ethanol and relatively high acidity at the end of the fermentation cycle in B3. The fact that the ethanol concentration at unload in this test was only 3.9 $g \cdot L^{-1}$ may have resulted not only in substrate deficiency, but also in an acetic acid concentration of 110 $g \cdot L^{-1}$, which may have concomitantly reduced the proportion of viable cells. This suggests the need to consider a potential inhibitory effect of acetic acid on cell growth.

Also, Figure 3 suggests the need to consider the presence of cell lysis in estimating the total concentration of cells. In fact, only cell lysis can account for the reduction in total cells near the end of each cycle.

4. Proposed model

The proposed model was developed on the basis of the above-described tests and experience gathered from previous models. Also, our model relies on the following starting assumptions:

(a) Non-viable biomass uses no substrate [6,7,16,19].

(b) The total cell concentration is the combination of those of viable and non-viable cells.

(c) Cell lysis, which was excluded in the above-described models, is an influential phenomenon in this context. Otherwise, the total cell concentration would increase steadily with time or level off at a given point during the process; in fact, as found in the above-described tests, such a concentration usually starts to decrease beyond a given point in time.

(d) Based on the previous experimental results, ethanol acts as a limiting substrate at low concentrations, but as a cell growth inhibitor at high levels.

(e) The acetification process is known to be limited by the oxygen supply to the medium. This is quite obvious since the fermentation rate can be raised by accelerating oxygen transfer in any way. This fact, and the ensuing limiting effect of this nutrient on cell growth, should therefore be considered in any kinetic equation intended to accurately describe the acetification process.

(f) As stated above and previously noted by other authors [7,10], acetic acid influences both cell growth and death.

The mass balance equations used, based on semi-continuous operation and the assumption of thorough mixing in the liquid phase, are as follows:

$$V\frac{dX_v}{dt}+X_v\frac{dV}{dt}=V\left(r_{X_c}-r_{X_d}\right) \tag{1}$$

$$V\frac{dX_d}{dt}+X_d\frac{dV}{dt}=V\left(r_{X_d}-r_{lisis}\right) \tag{2}$$

$$V\frac{dE}{dt}+E\frac{dV}{dt}=F_i\cdot E_i-V\cdot r_E \tag{3}$$

$$V\frac{dA}{dt}+A\frac{dV}{dt}=V\cdot r_A \tag{4}$$

$$V\frac{dO}{dt}+O\frac{dV}{dt}=F_i\cdot O^0+V\left[\beta\left(O^0-O\right)-r_O\right] \tag{5}$$

$$\frac{dV}{dt}=F_i \tag{6}$$

where X_v, X_d, E, A and O are the concentrations of viable cells, dead cells, ethanol, acetic acid and dissolved oxygen (all in $g\cdot L^{-1}$), respectively; V is the volume of the medium (L), F_i the raw material feed rate ($L\cdot h^{-1}$), E_i the concentration of ethanol in the fed raw material ($g\cdot L^{-1}$), O^0 that of dissolved oxygen in equilibrium with air ($g\cdot L^{-1}$), β a constant [see eq. (21)] encompassing factor K_La, the aeration flow rate and

volume (h^{-1}), r_{X_c} the cell growth rate (g cell·L^{-1}·h^{-1}), r_{X_d} the cell death rate (g cell·L^{-1}·h^{-1}), r_{lysis} the cell lysis rate (g cell·L^{-1}·h^{-1}), r_E the ethanol uptake rate (g ethanol·L^{-1}·h^{-1}), r_A the acetic acid (product) formation rate (g acetic acid·L^{-1}·h^{-1}) and r_O the dissolved oxygen uptake rate (g oxygen·L^{-1}·h^{-1}).

As can be seen, the previous mass balances consider not only the variation of the property in each equation by effect of its production or uptake, but also the concentration change by dilution resulting from the operating mode used. Also, the use of isothermal conditions avoids the need to formulate an energy balance.

The proposed expressions for the cell growth rate are as follows:

$$r_{X_c} = \mu_c \cdot X_v \tag{7}$$

$$\mu_c = \mu_{\max} \cdot f_e \cdot f_a \cdot f_o \tag{8}$$

where μ_c is the specific cell growth rate (h^{-1}); $\mu_{\max}$ its maximum value (h^{-1}); and f_e, f_a and f_o the terms representing the influence of ethanol, acetic acid and dissolved oxygen on cell growth.

Also, the terms used to weight the maximum cell growth rate are as follows:

$$f_e = \frac{E}{E + K_{SE} + \frac{E^2}{K_{IE}}} \tag{9}$$

$$f_a = \frac{1}{1 + \left(\frac{A}{K_{IA}} \right)^4} \tag{10}$$

$$f_o = \frac{O}{O + K_{SO}} \tag{11}$$

where K_{SE} is the ethanol saturation constant (g ethanol·L^{-1}), K_{IE} the ethanol inhibition constant (g ethanol·L^{-1}), K_{IA} the acetic acid inhibition constant (g acetic acid·L^{-1}) and K_{SO} the dissolved oxygen saturation constant (g oxygen·L^{-1}).

Like that proposed by Andrews [22], the equation for f_e (9) is a typical expression reflecting cell growth limitation and inhibition by the main substrate (ethanol). On the other hand, the proposed equation for f_a (10) only considers the potential inhibitory effect of acetic acid on cell growth since the boosting effect reported by some authors is certain to occur by virtue of the high acid levels always present in the fermenter. Finally, the equation for f_o (11) is a simple expression of the Monod type. As can be seen, the three terms can range from 0 to 1 provided the kinetic constants in their respective equations are all positive.

As can be inferred from eq. (8), the maximum level the specific growth rate can reach is $\mu_{\max}$, which is weighted by the terms reflecting the influence of the ethanol, acetic acid and dissolved oxygen concentrations (f_e, f_a and f_o, respectively). Consequently, as shown by eq. (7), the cell growth rate, r_{X_c}, is the mass of cells produced per unit time per unit volume by effect of the replication of viable cells —the only type of cell retaining this ability.

Based on the above-described experimental data, excessive concentrations of either ethanol or acetic acid can cause cell death. Therefore, both variables should be included in the kinetic expressions for cell death:

$$r_{X_d} = \mu_d \cdot X_v \tag{12}$$

$$\mu_d = \mu_d^0 \cdot f_{dE} \cdot f_{dA} \tag{13}$$

$$f_{dE} = 1 + \left(\frac{E}{K_{mE}} \right)^4 \tag{14}$$

$$f_{dA} = 1 + \left(\frac{A}{K_{mA}} \right)^4 \tag{15}$$

where μ_d is the specific cell death rate (h^{-1}), μ_d^0 is its minimum possible value (h^{-1}) and K_{mE} and K_{mA} are the ethanol and acetic-acid induced cell death rate constants (both in $g \cdot L^{-1}$), respectively.

The equation for μ_d considers a minimum specific cell death rate μ_d^0 due to cell ageing, which invariably occurs, and also the influence of the terms for the ethanol and acetic acid concentrations (f_{dE} and f_{dA}, respectively). Such terms are expressed in a similar manner and were near-unity (*i.e.*, they had no effect on natural cell death) at low ethanol or acetic acid concentrations. On the other hand, an increase in the ethanol and acetic concentrations of the medium resulted in an increasingly unfavourable medium that led to gradual death of the bacterial population.

Cell lysis is modelled with a first-order kinetic equation exclusively considering the concentration of non-viable cells:

$$r_{lysis} = \mu_{lysis}^0 \cdot X_d \tag{16}$$

where μ_{lysis}^0 is the specific cell lysis rate (h^{-1}).

Like that proposed by Romero *et al.* [16] the ethanol uptake is formulated as:

$$r_E = a_{E/X} \cdot r_{X_c} \tag{17}$$

coefficient $a_{E/X}$, which can be determined experimentally [19,23], is *ca.* 116.96 g ethanol$\cdot g^{-1}$ cell, and is the ethanol yield factor required to supply the amount of energy needed for biomass growth. Eq. (17) assumes that the amount of ethanol used to form ethyl acetate, that assimilated by the biomass and that used by the cells for maintenance are all negligible, as previously checked at an industrial level.

Similarly, acetic acid formation is formulated with equation:

$$r_A = \frac{r_E}{Y_{E/A}} \tag{18}$$

where $Y_{E/A}$ is the stoichiometric coefficient of ethanol uptake for acetic acid formation (0.767 g ethanol·g^{-1} acetic acid).

The influence of dissolved oxygen was formulated as follows:

$$r_O = \frac{r_E}{Y_{E/O}} \tag{19}$$

where $Y_{E/O}$ is the stoichiometric coefficient of ethanol relative to oxygen (1.44 g ethanol·g^{-1} oxygen). This equation was established under the assumption that the amount of dissolved oxygen used by cells for maintenance and that fixed as biomass were both negligible relative to the total oxygen uptake for the process.

The equation for oxygen supply to the fermentation medium was expressed with an equation previously proposed by Nieto [24]:

$$N_o = \beta\left(O^0 - O\right) \tag{20}$$

where N_o denotes the oxygen flow-rate (g oxygen·L^{-1}·h^{-1}) and β can be defined as:

$$\beta = \frac{K_L a}{1 + \frac{K_L a}{V \cdot V_m} \cdot \frac{RT}{H}} \qquad V \cdot V_m = \frac{Q}{V} \tag{21}$$

$K_L a$ being the overall volumetric coefficient of mass transfer for the liquid phase (h^{-1}), $V \cdot V_m$ the ratio of the air feed rate to the volume of the medium (h^{-1}), R the universal gas constant (0.082 atm·L·K^{-1}·mol^{-1}), T temperature (K), H the Henry constant (atm·L·mol^{-1}) and Q the air feed rate (L·h^{-1}).

Coefficient $K_L a$ was determined from a mass balance for dissolved oxygen in the medium [24]. In fact, using eqs. (5) and (21) on the assumption that the oxygen

concentration in the medium roughly levelled off when a steady state between oxygen supply and uptake was reached during the acetic acid production stage allowed the following expression to be formulated:

$$K_L a = \frac{1}{\frac{O^o - O}{r_O} - \frac{R \cdot T}{V \cdot V_m \cdot H}} \tag{22}$$

Substituting the dissolved oxygen concentrations and microbial oxygen uptake (r_O) values obtained in the above-described tests into this equation invariably led to a $K_L a$ value of *ca.* 500 h^{-1}. Therefore, the calculated value was assumed to vary insubstantially and be constant in all cycles as a result.

Eq. (20) is proposed instead of that more frequently used:

$$N_o = K_L a\left(O^* - O\right) \tag{23}$$

where O^* denotes the concentration of dissolved oxygen in equilibrium with air circulated through the medium ($g \cdot L^{-1}$). The reason for using eq (20) instead of eq. (23) is that O^* is variable and dependent on the extent to which oxygen in air is depleted; on the other hand, O^0 is known and constant: 7.6 $mg \cdot L^{-1}$.

As could be seen in part II, the new proposed equations seemingly make a proper representation of the process behaviour.

5. Conclusions

In this work, the state of the art in the mathematical modelling of the acetic acid fermentation process has been initially reviewed. Careful analysis of existing models for this purpose revealed discrepancies in the way the subject was approached depending on the experimental results obtained by their proponents. We then conducted a series of tests as a part of a more broad research work aimed at identifying the potential influence

of diverse operational variables in the process. Based on these results, a mathematical model for the acetic acid fermentation process used in the production of vinegar from white wine which considers the previously detected influences has been developed. The proposed model also relies to a variable extent in existing models which have been expanded to consider additional phenomena (*e.g.*, cell lysis) and exclude others. The ensuing kinetic equations have been formulated as simply as possible while retaining the ability to accurately model the influence of the major variables identified in the experiments.

The second paper in this series examines the practical identifiability and estimation of the model parameters, which are two essential, specially complex steps in validating any new mathematical model.

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Figures

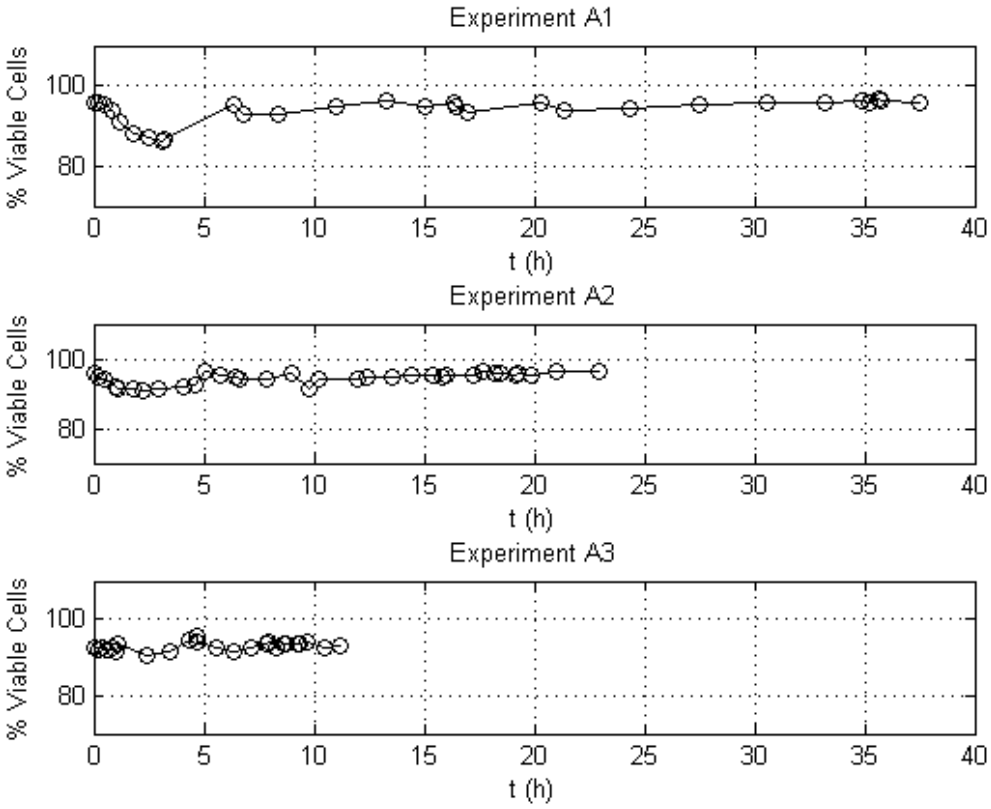

Figure 1: Average proportion of viable cells relative to total cells as determined in tests A1–A3.

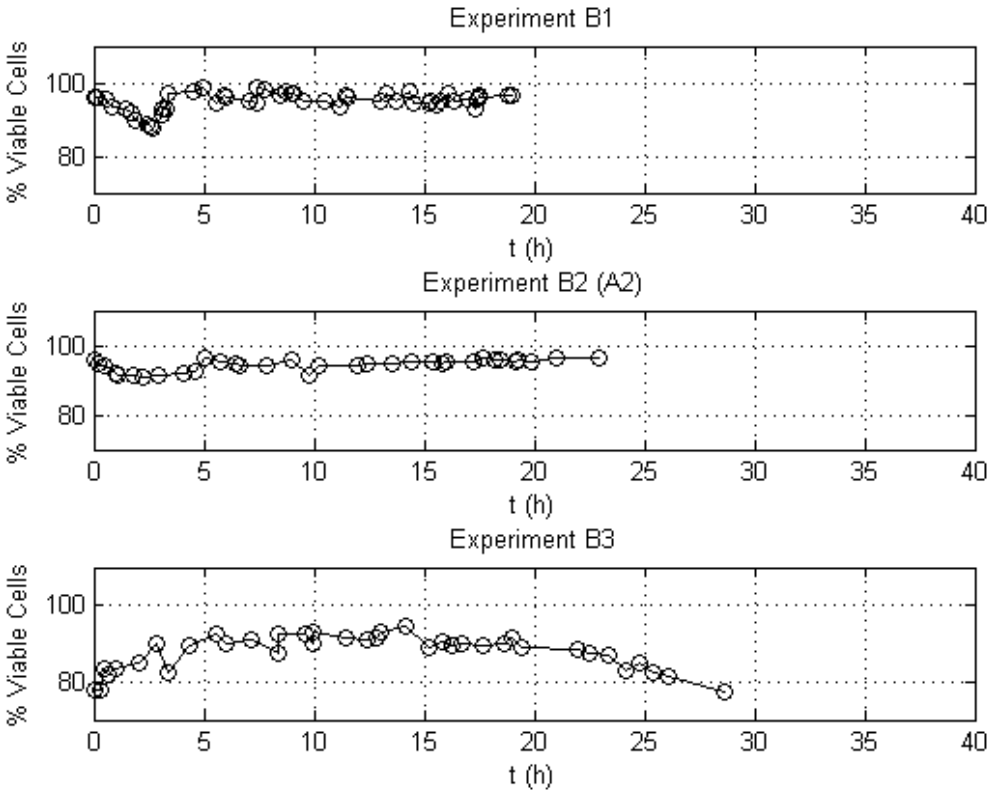

Figure 2: Average proportion of viable cells relative to total cells as determined in tests B1–B3.

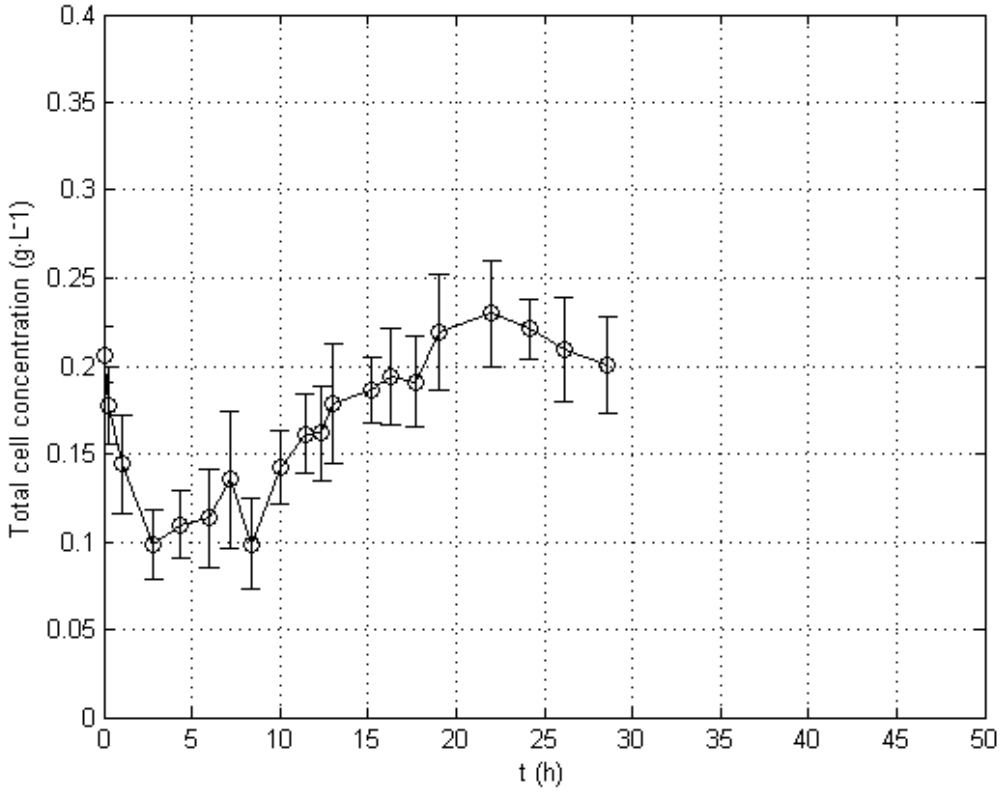

Figure 3: Temporal variation of the total concentration of cells in test B3.