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Optimum operating conditions in closed-system industrial acetifiers (batch operation): a study by computer simulation

M. Macías, I. Caro *, D. Cantero

Chemical Engineering Department, University of Cádiz, Aptdo. 40, Puerto Real, 11510 Cádiz, Spain

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Abstract

This work studies the optimum operating conditions in theoretical industrial acetifiers using computer simulation techniques. The fundamental part of the simulator is a global model for the growth of *Acetobacter aceti* in submerged culture compiled from the literature. The model is based on an expression which brings together a function for the specific rate of growth and another function for the specific rate of death, which reflects the combined influences of acetic acid, ethanol and oxygen. The kinetic parameters of the model are refitted by non-linear least-squares-type techniques, using data obtained from laboratory and industrial experimentation.

The optimum operating conditions have been calculated for two types of batch process: those with constant concentrations of dissolved oxygen and those with a constant rate of oxygen transfer to the fermentation medium. In both cases, the influence on the evolution of the process of different initial concentration levels of ethanol, acetic acid, biomass, dissolved oxygen and K_La has been studied.

Keywords: Computer simulation; Industrial acetifiers; *Acetobacter aceti*

1. Introduction

Generally, economic restrictions force industrial processes to work in a very small range of operating conditions. Some batch processes, such as acetic acid fermentation, have long operating times in each cycle and depend strongly on the operating variables. So it is very important to define the optimum conditions in order to achieve sufficient profitability. Kinetic simulators can be a highly appreciated tool for attaining these values and can reduce tests to eliminate extreme possibilities.

In this direction, acetic acid fermentation has been widely studied, both from the theoretical point of view as well as in its evolution in different types of fermenters. Consequently, a wide variety of models has been proposed for the kinetics of the process; these range from very simple models [1–6] to more complex global models [7–15], which take into account the activating and inhibiting effects of the substrate (ethanol and oxygen) and the product (acetic acid). However, none of these studies has put forward a general model sufficiently well developed to permit the design of a good simulator which is capable of performing simulations with batch acetifiers.

In this present paper, a global kinetic model is proposed compiled from the literature. The results have been sufficiently highly developed to facilitate simulation in closed systems and to enable the determination of the optimum operating conditions for batch acetification processes, with both a constant level of dissolved oxygen and a constant rate of oxygen transfer to the medium.

2. The simulation tool

2.1. The kinetic model

The basic equation on which the biomass growth model has been constructed is that proposed by Sinclair and coworkers [15–17]:

$$nX_v \xleftarrow{\mu_g} X_v \xrightarrow{\mu_d} X_n \qquad (1)$$

The quantity X_v of viable biomass grows at a specific rate μ_g of growth and suffers a process of loss of viability and death at a specific rate μ_d of death. The specific rates are defined as functions of the quantity of viable biomass and of the growth rates of the viable biomass, the non-viable biomass and the total biomass ($X = X_v + X_n$, where X is the total biomass concentration and X_n the non-viable biomass concentration):

* Corresponding author.

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$$\mu_g = \frac{1}{X_v}\left(\frac{dX}{dt}\right) \quad \mu = \frac{1}{X_v}\left(\frac{dX_v}{dt}\right)$$

$$\mu_d = \frac{1}{X_v}\left(\frac{dX_n}{dt}\right) \quad \mu = \mu_g - \mu_d \tag{2}$$

The expression for the microbial growth rate adopted in this model is that proposed by Romero et al. [18]:

$$\mu_g = \mu_m \frac{E}{E + K_{SE} + \left(\frac{E}{K_{IE}}\right)^2} \frac{1 + A/K_{SA}}{1 + (A/K_{IA})^3} \frac{O/K_{SO}}{1 + (O/K_{IO})^3} \tag{3}$$

E (g l^{-1}), A (g l^{-1}) and O (mg l^{-1}) represent the concentration levels of ethanol, acetic acid and dissolved oxygen respectively in the fermentation medium. The adjustment parameters for the growth function proposed by Romero et al. are as follows: $\mu_m = 0.22 \pm 0.02$ h^{-1}; $K_{SE} = 21.1 \pm 6.7$ g l^{-1}; $K_{IE} = 2.83 \pm 0.2$ g l^{-1}; $K_{SA} = 12.6 \pm 2.5$ g l^{-1}; $K_{IA} = 17.9 \pm 1.2$ g l^{-1}; $K_{SO} = 0.372 \pm 0.05$ ppm; $K_{IO} = 1.958 \pm 0.21$ ppm.

On the contrary, the expression for the specific rate of microbial death proposed by Mesa et al. [19] has been chosen:

$$\mu_d = \frac{K_M A^4}{E^3 + K_N} \tag{4}$$

However, the values given in the original paper are only valid for anoxic conditions; so they have had to be re-estimated, to allow this expression to be used for conditions where oxygen is present. In this case, the values obtained are as follows: $K_M = 0.138 \pm 0.03$ 1 g h^{-1}; $K_N = 2.55 \times 10^7 \pm 0.06 \times 10^7$ $g^3 l^{-3}$. This re-estimation was achieved by performing experiments analogous to the original experiments, but in which the concentration of oxygen in the medium was maintained at a constant level of between 1.0 and 8.0 ppm [20].

To summarize, therefore, the observed specific growth rate was defined as the difference between the two terms μ_g and μ_d, the former denoting the gross growth and the latter representing the loss of viability of the micro-organism.

Regarding the phenomena of consumption of substrate and formation of product, a simplified model has been selected, on the basis of the following considerations.

(1) The quantities of ethanol and oxygen assimilated by the biomass to synthesize cellular material are negligible compared with the total consumption of substrate by the energetic route (less than 0.5%). Equally, the consumption of substrate in the provision of the necessary energy for maintenance of the cells is negligible compared with the consumption needed for growth during fermentation [12,21].

(2) The consumption of ethanol and acetic acid in the formation of ethyl acetate by the chemical route is considered negligible, since it represents less than 3% of the total ethanol consumed in the process and, consequently, a smaller proportion of the total acetic acid produced [22].

(3) The losses of ethanol by evaporation have not been considered in this model. The experiments used to fit the parameters were performed in a closed system which eliminates any possible loss of volatile compounds by evaporation.

(4) The yield factors and stoichiometric coefficients are taken as constant throughout the entire fermentation process.

It follows therefore that the consumption of the principal substrates, ethanol and oxygen, as well as the formation of acetic acid, is exclusively a function of the growth of the micro-organism; the rates r_E and r_O of consumption of substrates and the rate of formation of the product r_A are represented by the following expressions:

$$-\frac{dE}{dt} = \frac{dX/dt}{Y_{X/E}} \quad \frac{dA}{dt} = \frac{-dE/dt}{Y_{E/A}} \tag{5}$$

constant-K_La processes:

$$dO/dt = K_La(O^* - O) - \frac{dX/dt}{Y_{X/O}}$$

constant-dissolved-oxygen processes:

$$O = \text{constant} \tag{6}$$

The estimated values for the two yield factors are $Y_{X/E} = 8.55 \times 10^{-3}$ (grams of biomass formed for each gram of ethanol consumed) and $Y_{X?O} = 1.23 \times 10^{-2}$ (grams of biomass formed for each gram of oxygen consumed). The stoichiometric coefficient for ethanol–acetic acid used is $Y_{E/A} = 0.767$ (grams of ethanol consumed for each gram of acetic acid formed).

All the coefficients compiled from bibliography, have been calculated for isothermal processes at 28 °C, and therefore the model devised is only applicable with reliability at around this temperature.

2.2. Simulation algorithms

The characteristics of the virtual batch fermenter developed with simulation purposes in this paper are as follows.

(1) The fermenter will operate isothermally at a a temperature of 28 °C.

(2) The transfer of oxygen to the fermentation medium can take place in two ways: (a) with a constant K_La throughout the duration of the fermentation process; (b) maintaining a fixed level of dissolved oxygen in the fermenter by means of an automatic control system.

(3) At the start of the fermentation process, the fermenter is considered to be fully loaded (volume) and it is kept constant during the entire process.

(4) The initial concentration levels of substrates, biomass and products, the characteristics volume of the fermenter and other operating conditions can be altered to different values in each simulation cycle.

(5) In the calculation algorithm, restrictions have been incorporated for all concentrations in the medium; these can

never be less than zero, to avoid physically unreal simulations.

2.3. Numerical method and step widths

The selection of the numerical method for the integration of the model has been made on the basis of an error propagation analysis and a convergence study. The selected method in this case has been the fourth-order Runge–Kutta method [23,24]. According to the convergence criteria the recommended step width for solve cases with constant dissolved oxygen is $\delta t = 0.1$ h.

However, because of the more rapid variability of μ_g owing to changes in oxygen concentration, the recommended step width for solve cases with constant $K_L a$ is $\delta t = 0.001$ h.

2.4. Parameter re-estimation

Principally because we have compiled equations from different authors in one single kinetic model, it is necessary to re-estimate the parameter values to secure the good fit of the system to real data. The experimental work carried out for re-estimation purposes has been done at a controlled temperature of 28 ± 1 °C. The fermenters used were two cyclindrical stirred tanks, with respective volumes of 5 l and 10 000 l. The 5 l fermenter was equipped with a gas recirculation system and a dissolved oxygen control system which enabled the set point to be easily maintained ($\pm 10\%$). This system consisted of an oxygen electrode and a PID controller which, by actuating a solenoid valve, allowed the recirculation gas to be enriched to the extent consumed. The 10 000 l fermenter, however, was fitted with an open aeration system for the oxygenation of the medium.

The fermentation medium used was a young wine of the Jerez wine-growing area, with the following characteristics: ethanol, 70–80 g l^{-1}, total acidity of tartaric acid, 0.5–1.0 g l^{-1}; sugar, 1–2 g l^{-1}; volatile esters, 1–5 mg l^{-1}; pH between 2.9 and 3.1.

The micro-organism used, in all cases, was a culture of *Acetobacter* used industrially in the production of vinegar in the area, which is preserved in our departmental collection classified as *Acetobacter aceti* UCA1.

Two series of five fermentations experiment each were carried out: the first in 5 l tanks with oxygen control (constant dissolved oxygen) and the second in 10 000 l tanks with $K_L a$ at constant levels (variable dissolved oxygen). Data corresponding to the latent phases were ignored since they were difficult to evaluate in the model under study.

The parameters of the model were re-estimated using non-linear least-squares-type analysis, which minimizes the differences between the experimental values of the adjustment variables and those obtained by simulation under the same conditions. Two adjustment variables were used: (a) time taken to achieve the highest growth rate of biomass; (b) the final value of acetic acid concentration.

In addition, parameters were divided into two groups of different physical significance. Included in the first group were all those parameters which directly affect the microbial growth (μ_m, K_{SE}, K_{IE}, K_{SA}, K_{SO} and K_{IO}). The second group of parameters correspond to those which strongly influence the microbial death (K_{IA}, K_M and K_N).

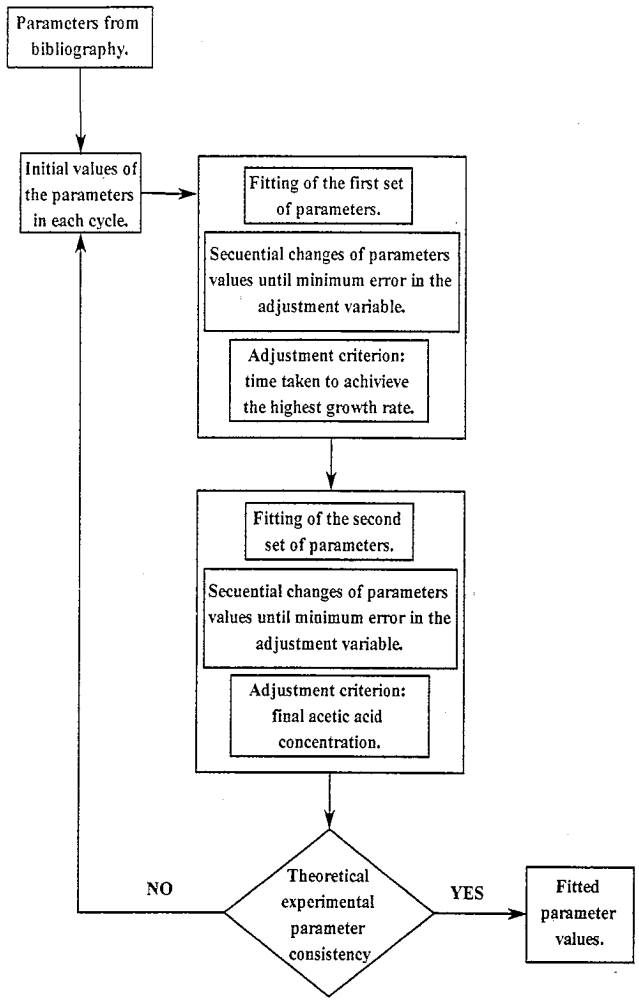

Fig. 1. Flow chart of the re-estimation method followed with the kinetic parameters.

Table 1
Re-estimated values for the parameters of the proposed kinetic model

$\mu_m = 0.235\ h^{-1}$	$K_{IA} = 29.1\ g\ l^{-1}$
$K_{SE} = 19.63\ g\ l^{-1}$	$K_M = 0.098\ l\ g^{-1}\ h^{-1}$
$K_{IE} = 3.026\ g\ l^{-1}$	$K_N = 2.5\ 10^7\ g^3\ l^{-3}$
$K_{SA} = 11.72\ g\ l^{-1}$	$Y_{X/E} = 8.55 \times 10^{-3}$
$K_{SO} = 0.346$ ppm	$Y_{E/A} = 0.767$
$K_{IO} = 2.09$ ppm	$Y_{X/O} = 1.23 \times 10^{-2}$

An iterative process (Fig. 1) was repeated with successive cycles until sufficient consistency was reached in the values obtained for the full set of parameters. The final values of the parameters obtained are exposed in Table 1.

The sensitivity of the adjustment variables used with respect to the parameters of the model is relatively large, given the complexity of the dependence and the large number of parameters included. A modification of $\pm 0.5\%$ in each parameter produces errors of approximately 3% in the value of the adjustment variables.

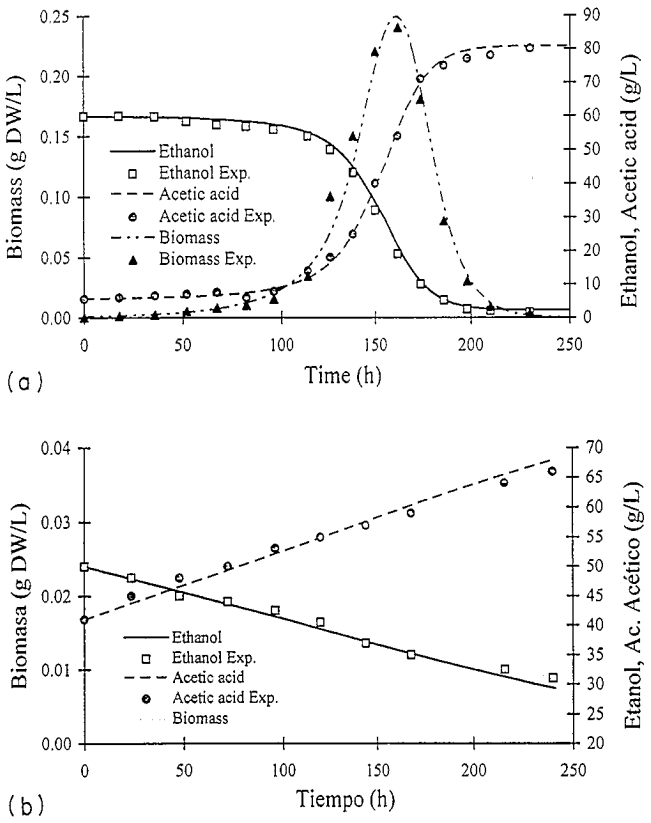

Fig. 2. Fit between an actual fermentation process and the simulation carried out: (a) 5 l fermenter. Initial acetic acid 5.7 g l^{-1}; initial ethanol 60 g l^{-1}; initial viable biomass 9.0×10^{-5} gDW l^{-1}; dissolved oxygen controlled at 4.5 ppm; (b) 10 000 l fermenter. Initial acetic acid 41.7 g l^{-1}; initial ethanol 50 g l^{-1}; initial viable biomass 1.8×10^{-2} gDW l^{-1}; constant K_La = 8.5 h^{-1}.

An example of the agreement obtained between actual fermentation processes and simulated data is presented in Fig. 2.

3. Simulation exercises

3.1. Simulation initial conditions

In order to study the acetification process, a series of virtual fermentations were carried out under different operating conditions and with different levels of substrate and product concentration. As an overall starting point of fermentation, some common initial conditions were set to all the cases.

The initial conditions correspond to those of an industrial fermentation process in which the inoculation phase has already taken place. For the wine acetification processes in the wine-growing area of Jerez, these conditions are as follows: ethanol, 70 g l^{-1}; acetic acid 2 g l^{-1} (originating in the inoculation phase); oxygen, 7.5 ppm (wine saturated as a result of decanting); total biomass, 40 million cells per millilitre; viable biomass 8 million cells per millilitre (the typical X_v in an inoculate of terminal phases from the batch acetification process).

The concentrations of the different constituents of the virtual wine used for the acetification processes were set at 80 g l^{-1} for alcohol and 0.5 g l^{-1} for acetic acid, these being typical values in the more rapid industrial processes.

3.2. Simulations for acetifications with constant K_La

3.2.1. General description

The analysis of the processes in which oxygen is not controlled was undertaken by means of a series of simulations with different values for the gas–liquid mass transfer coefficient K_La, while keeping the same initial conditions. For the general description of the process, two typical values for K_La in industrial fermenters were chosen (100 and 150 h^{-1}).

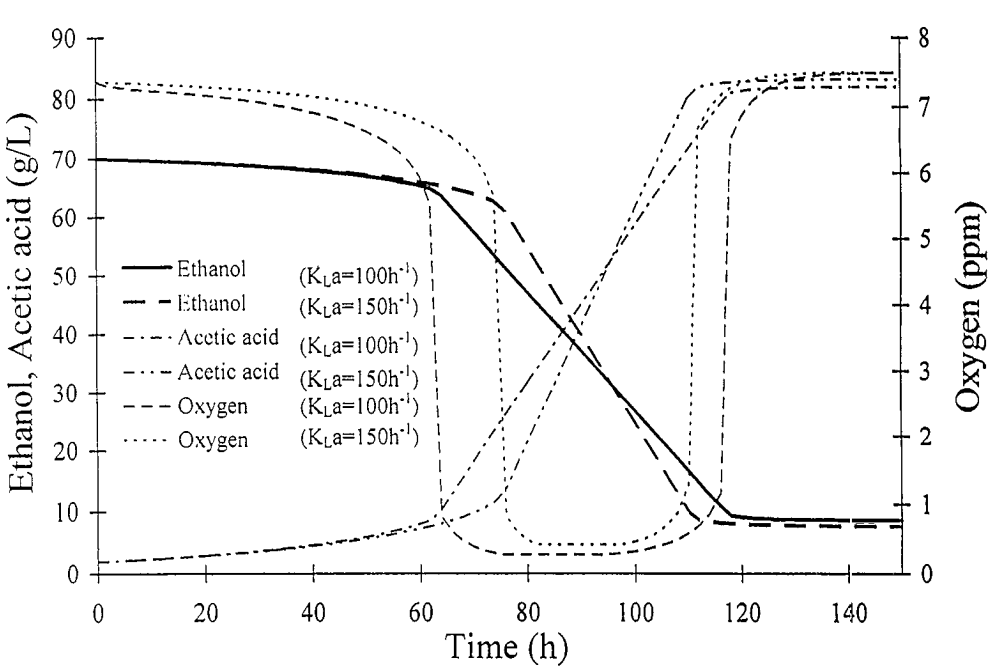

Fig. 3. Stimulation of batch fermentation with constant K_La (initial ethanol 70 g l^{-1}; initial acetic acid 0.5 g l^{-1}, initial dissolved oxygen concentration 7.5 ppm).

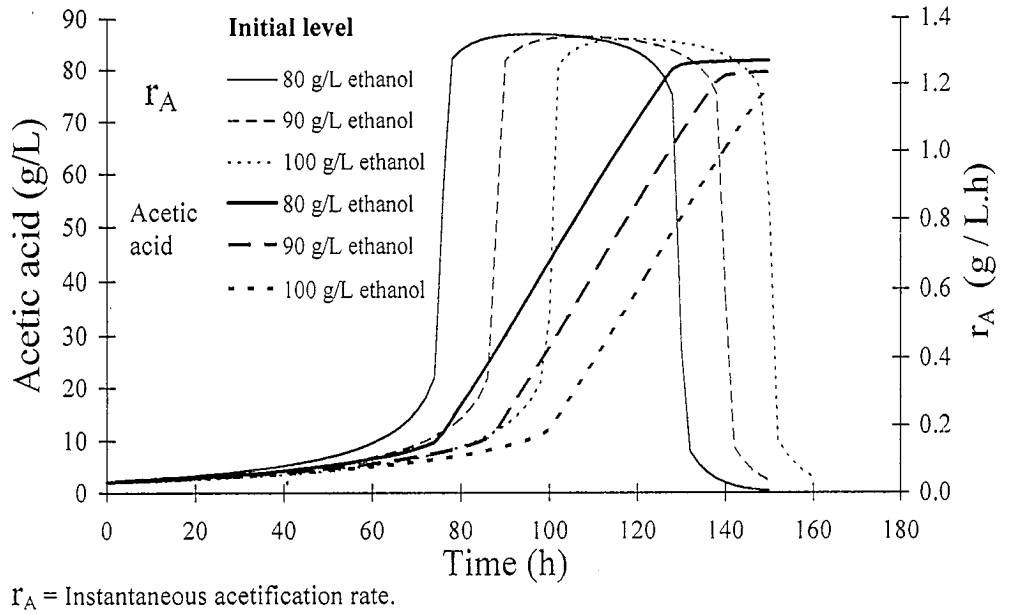

Fig. 4. Bath fermentation with constant K_La. Influence of the initial ethanol concentration. ($K_La = 100\ h^{-1}$; initial acetic acid 2 g l^{-1}).

The theoretical evolution of the concentrations of ethanol, acetic acid and dissolved oxygen in each case are shown in Fig. 3.

It can be seen that the evolution of the ethanol concentration in both cases is almost linear during the exponential phase, the rate of consumption increasing as K_La increases. It can also be observed that, when the rate of ethanol consumption is high, the concentration of dissolved oxygen falls to minimum values.

Finally, another notable fact is that commencement of fermentation occurs sooner with the lower value for K_La, although the degree of acidity obtained is independent of the value of the transfer coefficient. All these phenomena predicted by the simulation match very well the observed typical behaviours reported in the literature on acetification processes in general [4–10].

3.2.2. Influence of the initial ethanol content

In order to study the effect of the initial ethanol concentration on the evolution of the process general, simulations were performed with K_La setted at 100 h^{-1} and with different values for the initial ethanol concentration, from 80 to 100 g l^{-1}.

In principle, the rates of acetic acid formation do not show any apparent variation with the different values for initial ethanol content (Fig. 4), nor are any variations observed in

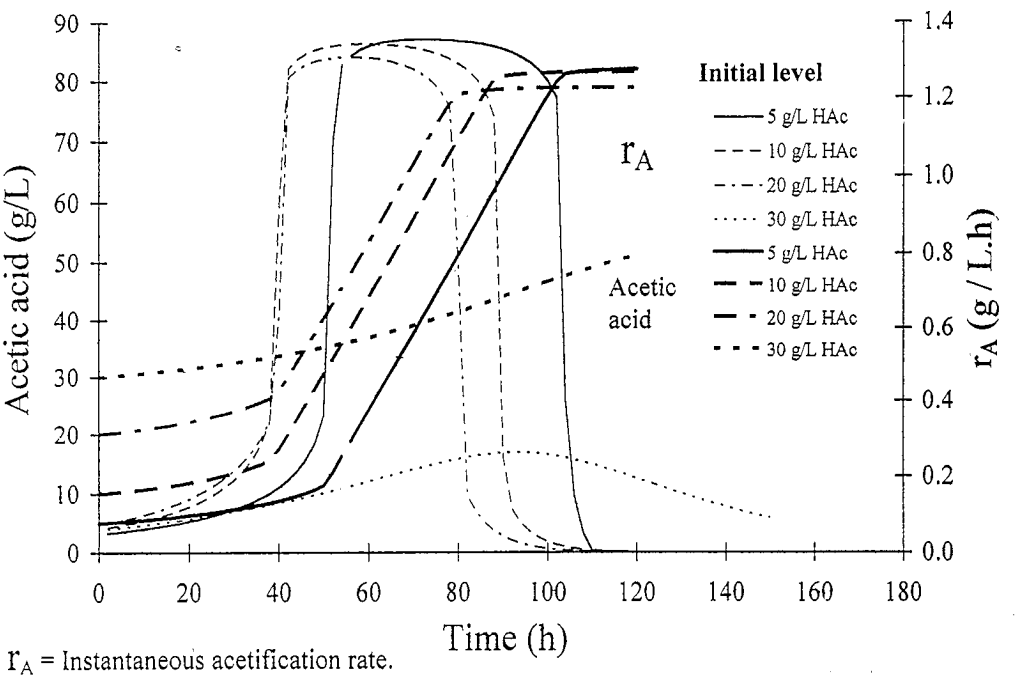

Fig. 5. Bath fermentation with constant K_La. Influence of the initial acetic acid concentration. ($K_La = 100\ h^{-1}$; initial ethanol 70 g l^{-1}).

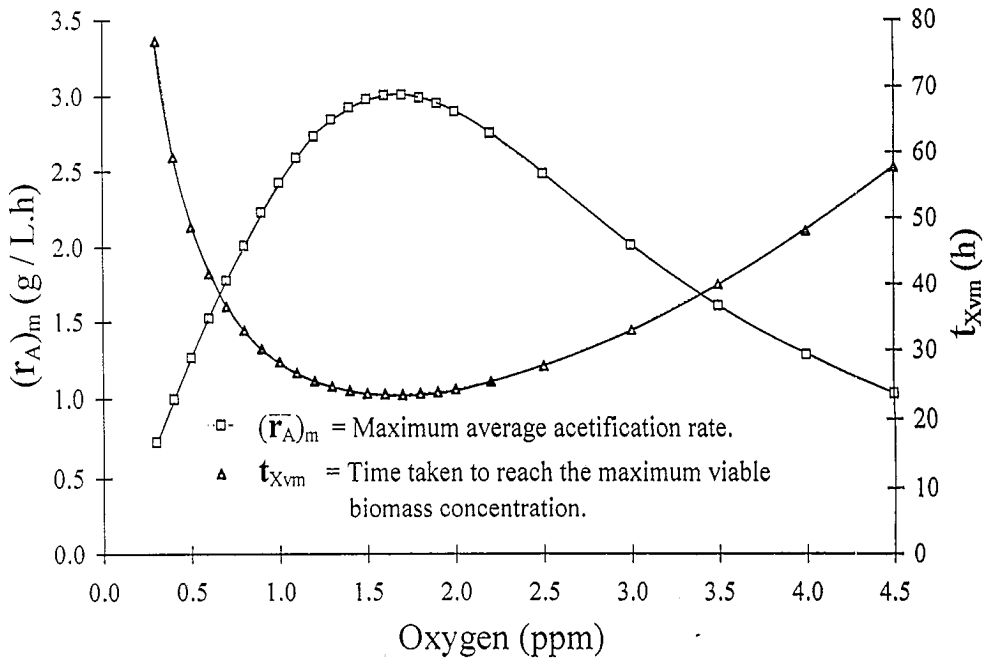

Fig. 6. Bath fermentation with constant dissolved oxygen. Influence of the dissolved oxygen concentration. (Initial ethanol 70 g l^{-1}; initial acetic acid 2 g l^{-1}).

the interval during which the exponential phase takes place. However, a dealy in the start of the process can be clearly noted. This delay is directly proportional to the initial ethanol concentration, clearly reflecting the inhibitory effect of the substrate. It should also be emphasized that the fermentation yield obtained does not appear to be significantly affected by the initial concentration of ethanol.

3.2.3. Influence of the initial acetic acid content

The influence of the initial acetic acid concentration was studied by modifying the level of concentration from 5 to 30 g l^{-1}, in processes with $K_La = 100\ h^{-1}$ and an initial ethanol concentration of 70 g l^{-1}.

From the results obtained (Fig. 5), it will be noted that the behaviour of the process with different initial concentrations of acetic acid is very variable. In particular, the wide variation existing between 20 and 30 g l^{-1} should be noted; this is shown as a reduction in the instantaneous acetification rate.

In addition, the activating effect of the acetic acid at low concentrations is clearly reflected [3,4]. Thus there is a significant reduction in the time taken for the inoculum to start the exponential phase, as the initial concentration of acetic acid is reduced. On this basis, the fastest process is that which begins with an acetic acid concentration of 15 g l^{-1}.

3.3. Simulation for acetification with constant dissolved oxygen content

3.3.1. Influence of the control value for dissolved oxygen

With the aim of estimating the optimum control value for dissolved oxygen, various simulations were undertaken with modifications in the oxygen present in the medium between 0.3 and 4.5 ppm, this being the range in which the specific growth rate has the most significant values.

Presented in Fig. 6 are the values of the maximum average acetification rate for each fermentative simulation and the times taken to reach the maximum levels of concentration of the viable biomass. A minimum time is achieved in acetifications carried out with between 1.0 and 2.0 ppm, and the time increasing greatly when the oxygen is reduced to below 1 ppm.

The strong dependence of the acetification rate on the concentration of dissolved oxygen is also clearly displayed, since it is possible to increase the rate by up to 100% by reducing the amount of dissolved oxygen in the process from 4 to 2 ppm.

The yields obtained also show an optimum oxygen level at between 1.5 and 2.0 ppm, although these levels of yield appear to be relatively unaffected by the oxygen in the medium. Thus the yield only increases from 88% with 4 ppm oxygen, to a maximum of 97% in the optimum working zone. This agrees with the fact that the dissolved oxygen has a great effect on the rate at which fermentation takes place, but a reduced influence on the depletion level of the substrate.

3.3.2. Influence of the initial ethanol content

Investigation of the influence of the initial concentration of ethanol on the fermentation process was carried out through a series of virtual fermentations with the oxygen content held constant at 2.0 ppm and the other initial conditions identical except for the ethanol concentration. This was modified to between 55 and 105 g l^{-1}.

Overall, the behaviour of the process is highly linear (Fig. 7); the greater the initial ethanol concentration, the longer is the time taken for the process and the slower is the rate of acetification.

The data on final acetic acid and the process yields clearly delineate two separate zones of operation: one highly efficient

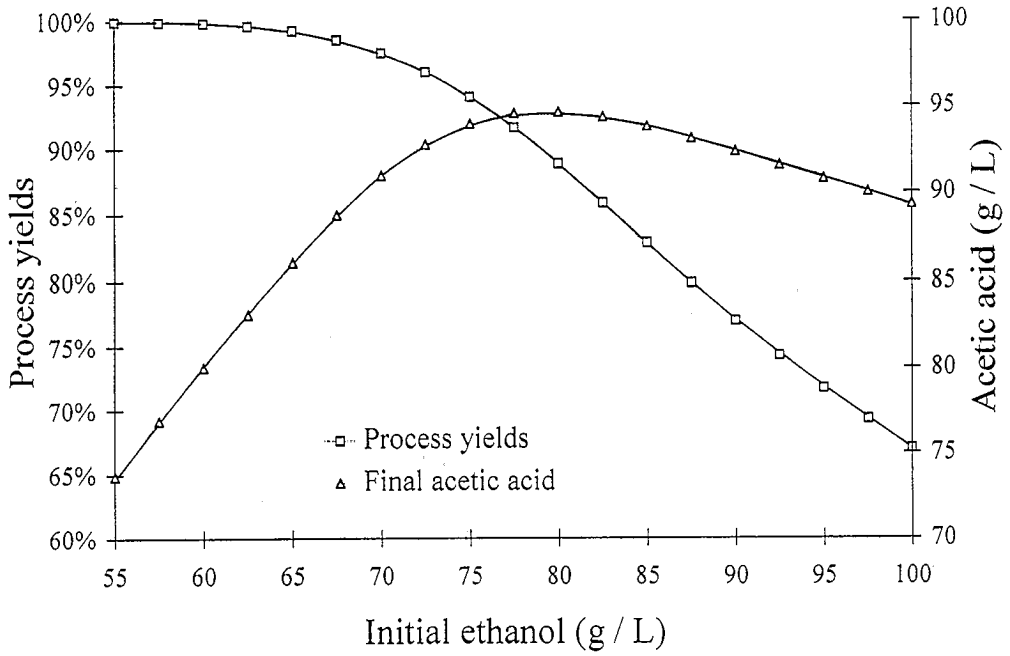

Fig. 7. Bath fermentation with constant dissolved oxygen. Influence of the initial ethanol concentration. (Dissolved oxygen 2 ppm; initial acetic acid 2 g l^{-1}).

up to an ethanol concentration of 75 g l^{-1}, where the substrate is completely depleted (yields of 100%), and a zone of low efficiency where the substrate is not fully depleted, since the inhibitory effect of the acetic acid and ethanol together result in a drop in viable biomass and therefore the premature halting of the fermentation process. When both curves are superimposed, it can be clearly seen that the optimum operating level is between 9.0 and 10.0 g l^{-1} of initial ethanol.

3.3.3. Influence of the initial biomass content

In order to analyse the influence of the initial biomass concentration on the evolution of the process, a series of initial concentrations of viable biomass spread across a wide range was chosen. The minimum value used was 10^{-5} g l^{-1}, a low value for a fermenter which has progressed beyond the latency phase; the maximum was 2.5×10^{-2} g l^{-1}, equivalent to 12 million cells per millilitre.

The results obtained revealed that the behaviour of the system, above the critical values of biomass (less than 0.005 g l^{-1}), can be considered as independent of the initial concentration of viable biomass. Even initial concentrations of biomass ten times higher produce only changes of 20% in the maximum average acetification rate, and in the time taken to achieve the greatest concentration of viable biomass.

One other interesting aspect revealed is that the processes finish with almost identical concentrations of substrate and product, independently of the initial concentration of viable biomass.

3.3.4. Influence of the initial acetic acid content

For the analysis of the influence of the initial acetic acid concentration on the evolution of the fermentation process, simulations were performed with increasing values for the initial acetic acid, starting from complete absence up to concentrations of 45 g l^{-1}.

The most notable result obtained (Fig. 8) is that, for values of initial acetic acid concentration greater than 45 g l^{-1}, almost no growth of the micro-organism occurs. Furthermore, the time taken to achieve the maximum concentration of viable biomass decreases as the initial acetic acid increases, until it reaches a minimum at an initial acid concentration of around 10 g l^{-1}; this agrees with the observed phenomenon of the activation of the acetification process with small initial quantities of acetic acid.

This same phenomenon determines the evolution of the maximum average acetification rate, whereby the maximum values appear at an initial acetic acid concentration of around 5 g l^{-1}.

It is significant that, although the microbial growth is greater at higher initial acetic acid concentrations below 10 g l^{-1}, the yield obtained diminishes as the initial acid concentration increases above 10 g l^{-1}.

This is because a high initial acid concentration effectively reduces the quantity of acid which is capable of being formed before values where the growth of the micro-organism is completely inhibited are reached.

To summarize, the best operating conditions are established at initial acetic acid concentrations of between 5 and 10 g l^{-1}.

4. Conclusions

This paper uses a general kinetic model for acetic acid fermentation under isothermal conditions in order to estimate the optimum operating conditions. The model can be applied across a wide range of concentrations of substrate (ethanol

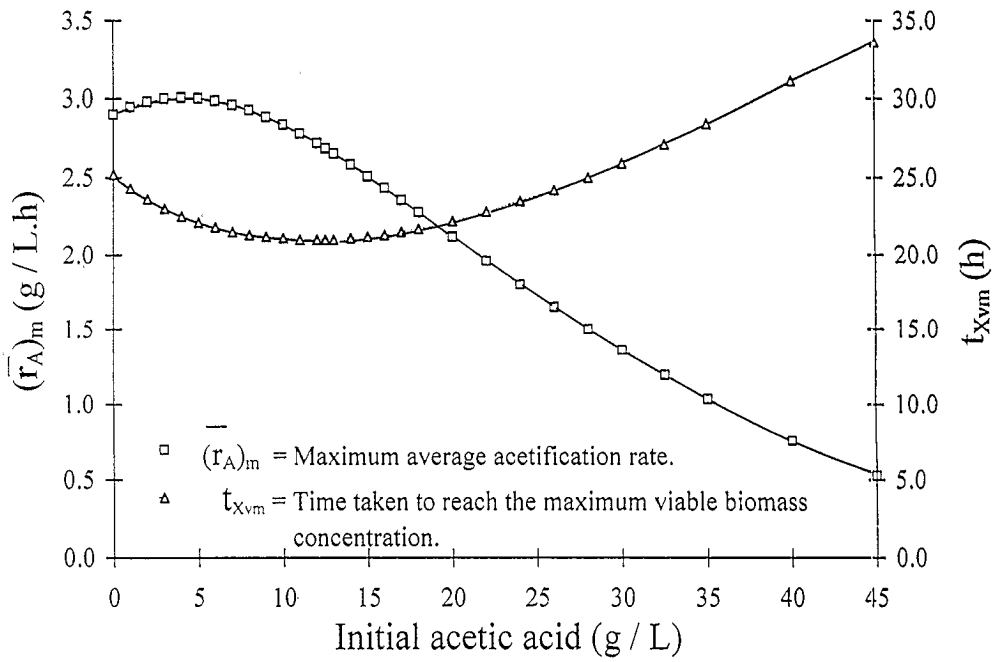

Fig. 8. Bath fermentation with constant dissolved oxygen. Influence of the initial acetic acid concentration (dissolved oxygen 2 ppm; initial ethanol 70 g l^{-1}).

and oxygen) and product (acetic acid) and therefore can provide a theoretical basis for the simulation of batch fermentation processes.

The method used to optimize the parameters of the model is based on actual experiments, resulting in good fits and a high degree of consistency.

The processes in which $K_L a$ is held constant require less process time than those in which the oxygen content of the medium is constant. It can also be observed that, in the former case, high initial concentrations of ethanol extend the duration of the process, while not exerting any appreciable influence over the final concentration of acetic acid formed. Equally, it can be stated that the optimum initial concentration of acetic acid lies between 10 and 15 g l^{-1}. In the case of processes with a controlled level of dissolved oxygen, optimum operation occurs with values for the oxygen content of between 1.5 and 2.0 ppm. The optimum initial concentration of ethanol is found to be between 75 and 80 g l^{-1}, these being the concentrations giving the greatest degree of final acidity with high values for rate of production and yield.

The initial viable biomass does not impact appreciably on the values for acidity obtained, nor on the yields; it does, however, affect the rate of acetification and the process time. Finally, the initial acetic acid content gives optimum operation at around 10 g l^{-1}.

Appendix A: Nomenclature

A acetic acid concentration (g l^{-1})
E ethanol concentration (g l^{-1})
K_{Ij} inhibition constant particular to species j (g l^{-1}, ppm)
K_M kinetic parameter for death rate (L g^{-1} h^{-1})
K_N kinetic parameter for death rate (g^3 l^{-3})
K_{Sj} saturation constant particular to species j (g l^{-1}, ppm)
O dissolved oxygen concentration (ppm)
O^* equilibrium oxygen concentration in the medium (ppm)
r_A instantaneous acetification rate (g l^{-1} h^{-1})
$(\overline{r_A})_m$ maximum average acetification rate (g l^{-1} h^{-1})
t time (h)
δt step width in the numerical integration (h)
t_{XVm} time taken to reach the maximum viable biomass concentration (h)
X total biomass concentration (g DW l^{-1})
X_n non-viable biomass concentration (g DW l^{-1})
X_v viable biomass concentration (g DW l^{-1})
$Y_{E/A}$ ethanol/oxygen stoichiometric coefficient
$Y_{X/E}$ biomass/ethanol yield factor (g DW (g ethanol)$^{-1}$)
$Y_{X/O}$ biomass/oxygen yield factor (g DW (g oxygen)$^{-1}$)

Greek letters

μ observed specific growth rate (h^{-1})
μ_d specific death rate (h^{-1})
μ_g gross specific growth rate (h^{-1})
μ_m maximum specific growth rate (h^{-1})

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