A kinetic model for growth of *Acetobacter aceti* in submerged culture

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(Received June 10, 1993; in final form November 1993)

Abstract

This paper proposes a kinetic model for growth, in low alcohol media, of *Acetobacter aceti* in submerged culture. The model combines the influence of substrate (ethanol) concentration, product (acetic acid) concentration and dissolved oxygen on the specific microorganism growth rate. Experimental data were obtained, both in the laboratory and industrially, using a variety of discontinuous fermentation apparatus with automatic control, and either open or closed gas recirculation systems. The operation conditions applied were those typical of acetic fermentation processes in the food industry. Examination of the results obtained makes it possible to determine the characteristic influence of each of the variables indicated, and to calculate the values of the different parameters of the proposed growth equation.

1. Introduction

In the literature a large number of kinetic models for microbial growth applicable to very different fermentation processes, are described. Some of the models are of highly general character (exponential, logistic, Monod, *etc.*) while others are of more particular application. In a considerable number of industrial processes, general growth models can be applied with sufficient accuracy, however, because of the particular features of acetic fermentation, this process requires a very specific model.

In this sense the following, from the microbial growth standpoint, are the most important features of acetic fermentation.

A clear substrate inhibition action (ethanol) is noted at concentrations in excess of 50 g L^{-1}. Growth rates at concentrations above 150 g L^{-1} are very low [1].

Although acetic fermentation is an aerobic process, oxygen is also seen to have an inhibiting effect for concentrations above approximately 5 p.p.m. of dissolved oxygen, considerably less than the saturation value [2].

The product (acetic acid) is also seen to produce an inhibiting effect at concentrations over 20 g L^{-1}, though it has a slight enhancing action at lower concentrations. In this way, when using media with a certain initial acid concentration, the process rates obtained are higher than those when the acid is absent [3].

The general features of industrial acetic fermentation processes are as follows.

There is a diversity of gas–liquid contact systems, whether in a discontinuous regime (*eg.* the Orleans, Luxembourg or Frings method) or in a continuous regime (*eg.* the Schutzenbach method, ventilated tower or bubble column). Each system offers very different values for oxygen mass transfer coefficients.

Generally, complex natural media provide the fermentative basis, with ethanol content as the main carbon source (between 50 and 120 g L^{-1}), and bacterial inocula (usually of the *Acetobacter* genus) are selected according to their fermentation properties.

Bisulphite ions are usually added to the fermentation medium as antioxidant and antiseptic, in concentrations of up to 100 p.p.m. The growth of this *Acetobacter* is significantly influenced only at higher SO_2 concentrations [4].

In most processes restricted dissolved oxygen conditions are usually applied, since economically acceptable aeration rates must be provided (0.05–0.10 v.v.m.). In this sense it is verified that concentrations of the order of 10–20% of saturation may be considered not to limit the overall acetification rate [5]. The medium must be oxygenated continuously; interruptions of more than 30 s may be enough to inactivate most of the bacterial population [6].

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SSDI 0923-0467(93)06046-S

Finally, the general characteristics of the microorganism responsible for acetification are as follows.

In terms of pH, acetic bacteria may be said to show optimal growth in the pH range between 5.0 and 6.5 [7], although they show great capacity to survive at a pH of between 3 and 4 [8].

In the case of the *Acetobacter aceti* species, the maximum working temperature is between 30 and 35 °C [9]. However, temperatures are usually lower in industry to avoid heating costs and limit the loss of volatile compounds via evaporation caused by the aeration.

The nutritional demands of the acetic bacteria are limited, their most restrictive requirements are for nitrogenated compounds, phosphates and some B_9 complex vitamins.

Most organic and inorganic acids can be said to have an inhibiting effect on the process [6].

It is generally well known that, in this type of fermentation, dissolved oxygen concentration is a decisive factor in the overall rate of the process. However, in dealing with the kinetic modelling of acetic fermentation, the presence of oxygen-related parameters is usually avoided. Models which include this variable have thus not been proposed, in quantitative form, using kinetic equations.

2. Present knowledge

As for most fermentative models which include microbial growth, acetic fermentation is based on the consideration that the biomass present in the medium is either viable (denoted X_v) or non-viable (denoted X_n). The total biomass X present is the sum of these two quantities, and absolute or specific temporal variations of biomass may also be additive quantities.

$$X = X_v + X_n$$

$$\frac{dX}{dt} = \frac{dX_v}{dt} + \frac{dX_n}{dt}$$

$$\frac{1}{X_v}\frac{dX}{dt} = \frac{1}{X_v}\frac{dX_v}{dt} + \frac{1}{X_v}\frac{dX_n}{dt} \quad (1)$$

$$\mu_g = \mu + \mu_d$$

$$\mu = \mu_g - \mu_d$$

Therefore, the observed specific growth rate μ in a culture is the difference between the overall specific growth rate μ_g and the overall specific death rate μ_d of biomass, of course with the assumption that only the viable biomass is able to multiply. In general, the influence of the different operative variables on growth and death rates is modelled separately for the two phenomena, as their effect may differ in each case.

One of the most recently published models for μ_g, provided by Nanba *et al.* [3], incorporates the influence of the ethanol and acetic acid concentrations on the specific growth rate of *Acetobacter aceti*. This model proposes a particular expression of non-competitive inhibition to reflect the inhibiting effect of the ethanol. However, the influence of the product is expressed on the basis of a specific enzymatic action model: the cooperative effects model of the special inhibition–activation type [10].

Relative to the cellular death phenomena and working with a continuous acetic fermentation system, Park *et al.* [2] proposed a direct ratio between the overall specific death rate and the acetic acid concentration raised to the power of four. However, Bar *et al.* [11] proposed an exponential ratio between these variables for discontinuous *Acetobacter aceti* cultures.

Some of the existing general growth models described in the literature are applicable only in limiting oxygen conditions [1, 5], while others apply only in non-limiting conditions [3]. None, however, reflects the overall action of the oxygen throughout the range of operation. Therefore, this study deals with the development of a growth model for *Acetobacter aceti* which incorporates the combined influence of the three significant variables referred to above (ethanol, acetic acid and dissolved oxygen) throughout the entire range of operational conditions. The aim of this work is to provide a more general model of greater utility, particularly for application in processes with variable oxygen concentrations which are the standard when external oxygen control is not established.

3. Materials and methods

To study the growth rate of the microorganism responsible for acetification from the kinetic point of view, a number of acetic fermentation experiments were carried out, in discontinuous equipment at the controlled temperature of 26 ± 1 °C. This is the average industrial process temperature in mild zones. All cases used a submerged culture of one of the main vinegar-isolated strains, classified as *Acetobacter aceti* ATCC 15973.

Two scales of operation were studied, one involving a 5 L stirred tank fermenter, the other a 10 000 L stirred tank fermenter. The equipment was operated with aeration rates between 0.05 and 0.5 v.v.m., and a stirring rate of 400 rev min^{-1}.

Oxygen transfer coefficients, from the gas to the liquid phase, were determined by the static absorption–desorption method [12]. They proved to be over 100 h^{-1} in all systems used for the experiments, ensuring the supply of oxygen required for biomass under the different operational conditions.

The fermentative medium used in all the experiments was a complex natural medium: a young wine from the Jerez winemaking area having 70–90 g L^{-1} ethanol; total acidity 15–20 g L^{-1} tartaric acid; 1–2 g L^{-1} sugars; 0.5–1.0 g L^{-1} high alcohols; 1–5 mg L^{-1} volatile esters; pH 2.9–3.1; 60–70 mg L^{-1} sulphur dioxide. This medium was sterilised at 120 °C for 20 min and its pH was subsequently established at 4, with 1 M KOH, to provide the most suitable conditions for growth of the microorganism.

The general layout of the fermentation equipment used in the different experiments is shown in Fig. 1. The fermenters were provided with a gas recirculation system making it possible to operate either in a closed system, preventing the escape of volatile compounds from the gaseous outlet flow, or else in an open system. To offset the oxygen consumption by the biomass, pure oxygen was injected discretely into the recirculation flow to the fermenter. The system had a dissolved oxygen electrode and a controller which were used to maintain the dissolved oxygen at the required level. The experiments proceeded at a wide variety of values of this parameter within the range possible for the ethanol concentration (0–8 p.p.m.) and temperature conditions. The range of the dissolved oxygen signal error was maintained at ±10% of the set point.

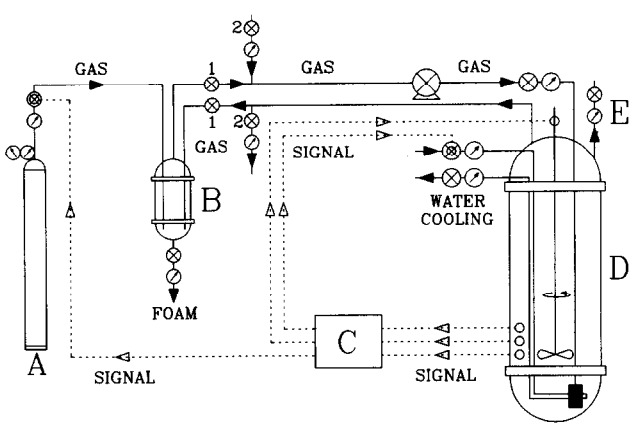


Fig. 1. Scheme of the experimental equipment used. A oxygen supply unit, B gas mixer and foam trap, C automatic control equipment, D discontinuous aerated fermenter, E pressure safety value. Open gas circulation system, valves 1 closed and 2 open. Gas recirculation system, valves 1 open and 2 closed.

The fermenters, containing the sterile fermentative medium, were inoculated in all cases by the addition of 10% (by volume) of a previously prepared inoculum. This inoculum consisted of an identical medium presenting a high *Acetobacter aceti* growth rate, obtained by parallel acetic fermentation processes in incubation chambers. Before each inoculation it was verified that the operational variables were stable and the control elements were operating correctly.

To monitor the different variables during the fermentation processes, fermenter samples were taken at regular time intervals and the following assays were carried out. Ethanol and acetic acid concentration using gas chromatography [13]; viable biomass concentration by plate count in a YEPD (10% yeast extract, 20% peptone, 20% D-glucose) medium [14]; total biomass concentration by a Neubauer chamber count [15]. The appropriate equipment was used to continuously monitor pH, dissolved oxygen concentration and temperature of the fermentation medium.

In order to maintain a certain dimensional homogeneity in the equations of the models, total and viable biomass concentration need to be expressed in gDW L^{-1} whereas the analytical procedures used in this study provide these concentrations in CFU mL^{-1}. It was therefore necessary to first calculate the correlation between the two dimensions. This correlation was established by simultaneously measuring, in a large number of samples, the quantity of total biomass using two different procedures: the Neubauer chamber cell count (CFU mL^{-1}) and the dry weight of biomass (gDW L^{-1}). The results are shown in Fig. 2.

Following the methodology described above, a series of discontinuous acetic fermentation experiments was carried out. The experiments can be grouped according to the operational conditions and the range of values covered by the different variables throughout the process, as outlined below.

Six experiments in the 5 L fermenter with an open gas circulation system. The ranges of the different variables observed during these experiments were as follows. Dissolved oxygen 7–8 p.p.m.; ethanol 70–20 g L^{-1}; acetic acid 20–70 g L^{-1}; total biomass 0.01–0.10 g L^{-1}; viable biomass 1×10^{-6}–1×10^{-3} g L^{-1}.

Five experiments in the 5 L fermenter with a closed gas recirculation system. The ranges of the variables were as follows. Dissolved oxygen 1–7 p.p.m.; ethanol 70–10 g L^{-1}; acetic acid 10–80 g L^{-1}; total biomass 0.05–0.50 g L^{-1}; viable biomass 1×10^{-6}–1×10^{-3} g L^{-1}.

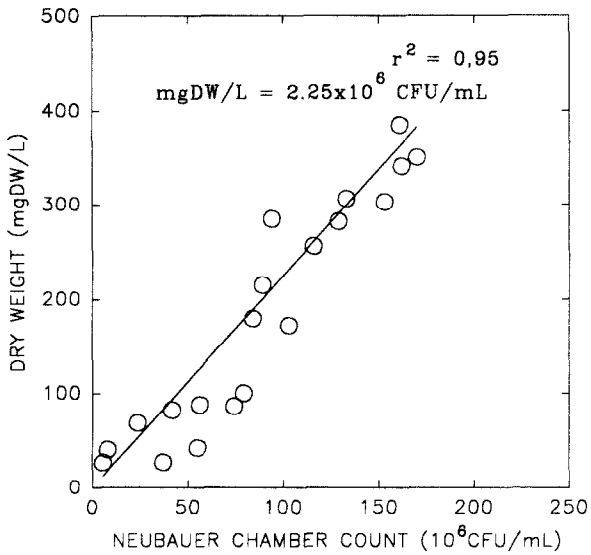


Fig. 2. Correlation between the two assays of biomass concentration used, milligrams of dry weight per litre (mgDW L^{-1}) and colony forming units per millilitre (CFU mL^{-1}).

Six experiments in the 10 000 L fermenter with an open gas circulation system. In this case the ranges of the variables were: dissolved oxygen 0–1 p.p.m.; ethanol 60–20 g L^{-1}; acetic acid 30–80 g L^{-1}; total biomass 0.01–0.20 g L^{-1}; viable biomass 1×10^{-6}–1×10^{-3} g L^{-1}.

4. Experimental results and discussion

4.1. Calculation of specific microbial growth rates

Using the experimental data of total biomass X_i and viable biomass X_{vi} at time t_i, the value of the overall specific growth rate μ_{gi} at that time can be estimated. The procedure to be followed may be based on numeric or graphic differentiation methods, depending on the type and number of accumulated experimental data. In this case, a numeric differentiation procedure was followed in accordance with the following calculation algorithm.

$$\mu_{gi} = \left(\frac{dX}{dt}\right)_i \frac{1}{X_{vi}}$$

$$\left(\frac{dX}{dt}\right)_i = \frac{1}{2}\left(\frac{X_i - X_{i-1}}{t_i - t_{i-1}} + \frac{X_{i+1} - X_i}{t_{i+1} - t_i}\right) \qquad (2)$$

The results obtained represent the set of specific rates μ_{gi} for different concentrations E_i of ethanol, A_i of acetic acid, and O_i of dissolved oxygen, measured at each time t_i. Figure 3 shows the values of this specific rate, plotted in relation to the three variables under examination, for the three complete sets of experiments carried out. These data were used to study the influence of each of the variables upon microbial growth.

4.2. The influence of ethanol on growth

With reference to the individual influence of substrate on microbial growth under fixed acetic acid and oxygen conditions, a particular equation of the substrate inhibition type is proposed. This equation is in accordance with considerations previously mentioned in this study, and information from the literature published on the matter [1, 3]. Thus, specifically, the equation proposed for the individual influence of ethanol on the specific overall growth rate is as follows.

$$\mu_{gE} = \mu_{maxE}\left(\frac{E}{E + K_{SE} + \left(\frac{E}{K_{IE}}\right)^2}\right) \qquad (3)$$

Where the values of the parameters must be situated within specific intervals in response to the following experimental factors: the maximum growth rate is obtained for values of E of about 15 g L^{-1}; the growth rate drops to 20% of the maximum value for ethanol concentrations around 70 g L^{-1}. The ranges of the parameters corresponding to these considerations are: K_{SE} = 10–30 g L^{-1} and K_{IE} = 2–4 g L^{-1}. Moreover, the parameter μ_{maxE} must incorporate the influence of acetic acid and oxygen, and acquires a different constant value for each pair of values of those variables.

In eqn. (3), K_{SE} can be considered a substrate saturation constant, so reflecting the metabolic affinity of the microorganism for it. Low values of the constant indicate a high microorganism–substrate affinity. On the other hand, constant K_{IE} quantifies the inhibiting action of the substrate on growth (inhibition constant). In this case, low values for the parameter indicate a high level of substrate inhibition.

4.3. The influence of acetic acid on growth

To study the influence of the fermentation product on growth rate, with fixed conditions of ethanol and dissolved oxygen, an equation of the activation–inhibition by-product type is proposed. This type agrees with the particular effect of activation of growth detected for small acetic concentrations [3]. Analogous equations have been applied to special cases of enzymatic kinetics of similar kinetic behaviours [10]. In our case, the proposed equation is as follows.

$$\mu_{gA} = \mu_{maxA}\left(\frac{1+\frac{A}{K_{SA}}}{1+\left(\frac{A}{K_{IA}}\right)^3}\right) \quad (4)$$

The values of the parameters introduced must adjust to the following experimental circumstances: the maximum growth rate is obtained for values of A around 10 g L^{-1}, this rate is of the order of 20% greater than that obtained in the absence of acetic acid and is reduced to 10% of the maximum value for acid concentrations of around 50 g L^{-1}. The ranges for the parameters corresponding to these considerations are: K_{SA} = 10–15 g L^{-1} and K_{IA} = 15–20 g L^{-1}. In this case, the parameter μ_{maxA} must include the influence of the ethanol and oxygen, and it also acquires a different constant value for each pair of values of such variables.

The value of the coefficient K_{SA} is an indicator of the degree to which the acetic acid enhances the growth of the acetic bacteria (activation constant), while the parameter K_{IA} shows the inhibiting effect of the acid (inhibition constant).

4.4. The influence of oxygen on growth

Finally, for the individual influence of oxygen, a special inhibition equation is proposed which reflects the effect observed on growth for concentrations close to saturation [2]. The following equation is proposed for this case

$$\mu_{gO} = \mu_{maxO}\left(\frac{\frac{O}{K_{SO}}}{1+\left(\frac{O}{K_{IO}}\right)^3}\right) \quad (5)$$

In this equation there are in fact only two independent parameters, μ_{maxO}/K_{SO} and K_{IO}. However, three parameters are included in order to maintain the analogy with eqns. (3) and (4). Thus K_{SO} is actually defined according to the value obtained for μ_{maxO}/K_{SO} and to the corresponding value assigned to μ_{maxO}.

In this sense, the following circumstances must be observed for the values of these parameters: the maximum growth rate is obtained at oxygen values of between 1 and 3 p.p.m. and the rate drops to 10% of the maximum value for oxygen concentrations around saturation (8 p.p.m.). The range of the parameters corresponding to these considerations are μ_{maxO}/K_{SO} = 3–5 h^{-1} p.p.m.$^{-1}$ and K_{IO} = 1–3 p.p.m. The parameter μ_{maxO}/K_{SO} must, in this case, include the influence of the ethanol and acetic acid, and it acquires a different constant value for each pair of values for those variables.

The physical meaning acquired by the parameters K_{SO} and K_{IO} is similar to those of the previous cases, but now referred to values for dissolved oxygen.

4.5. The combined influence of the variables

From the above considerations, a general equation can be proposed for the specific overall growth rate. If it is assumed that there are no significant synergistic effects between the variables in question (*i.e.* that the overall effect observed is the product of the individual influences) then the general equation takes the following mathematical form.

$$\mu_g = \mu_{max}\left(\frac{E}{E+K_{SE}+\left(\frac{E}{K_{IE}}\right)^2}\right) \times \left(\frac{1+\frac{A}{K_{SA}}}{1+\left(\frac{A}{K_{IA}}\right)^3}\right)\left(\frac{\frac{O}{K_{SO}}}{1+\left(\frac{O}{K_{IO}}\right)^3}\right) \quad (6)$$

Where μ_{max}, K_{SE}, K_{IE}, K_{SA}, K_{IA}, K_{SO} and K_{IO} are characteristic parameters of the microorganism, and may also be influenced to a greater or lesser degree by the type of substrate (pH, nutrients, inhibitors, *etc.*) and by the temperature.

In eqn. (6), in a manner analogous to eqn. (5), μ_{max}/K_{SO} is a single adjustable parameter. If μ_{max} acquires the physical meaning of representing the maximum observable value of μ_g obtained for a given concentration E_{max} of substrate, A_{max} of acetic acid and O_{max} of dissolved oxygen, then K_{SO} is also invariably defined.

The proposed equation can be justified theoretically if the following considerations are made. In the first place, it is considered that the growth of the biomass is a process which depends, in energy terms, on the substrate metabolism; the greater the rate at which the cell metabolizes the ethanol, the greater the rate of specific growth. In the second place, it is considered that this metabolism comprises a chain of reactions regulated by enzymes (alcohol–dehydrogenase, aldehyde–dehydrogenase, *etc.*) and co-enzymes (mainly NADH) [16, 17], so that the overall process must take place at the velocity of the slowest step, and this velocity is related to the concentration of the controlling enzyme.

In addition, to establish a direct relation between the growth rate and enzymatic reaction, it is necessary to consider the following approximations.

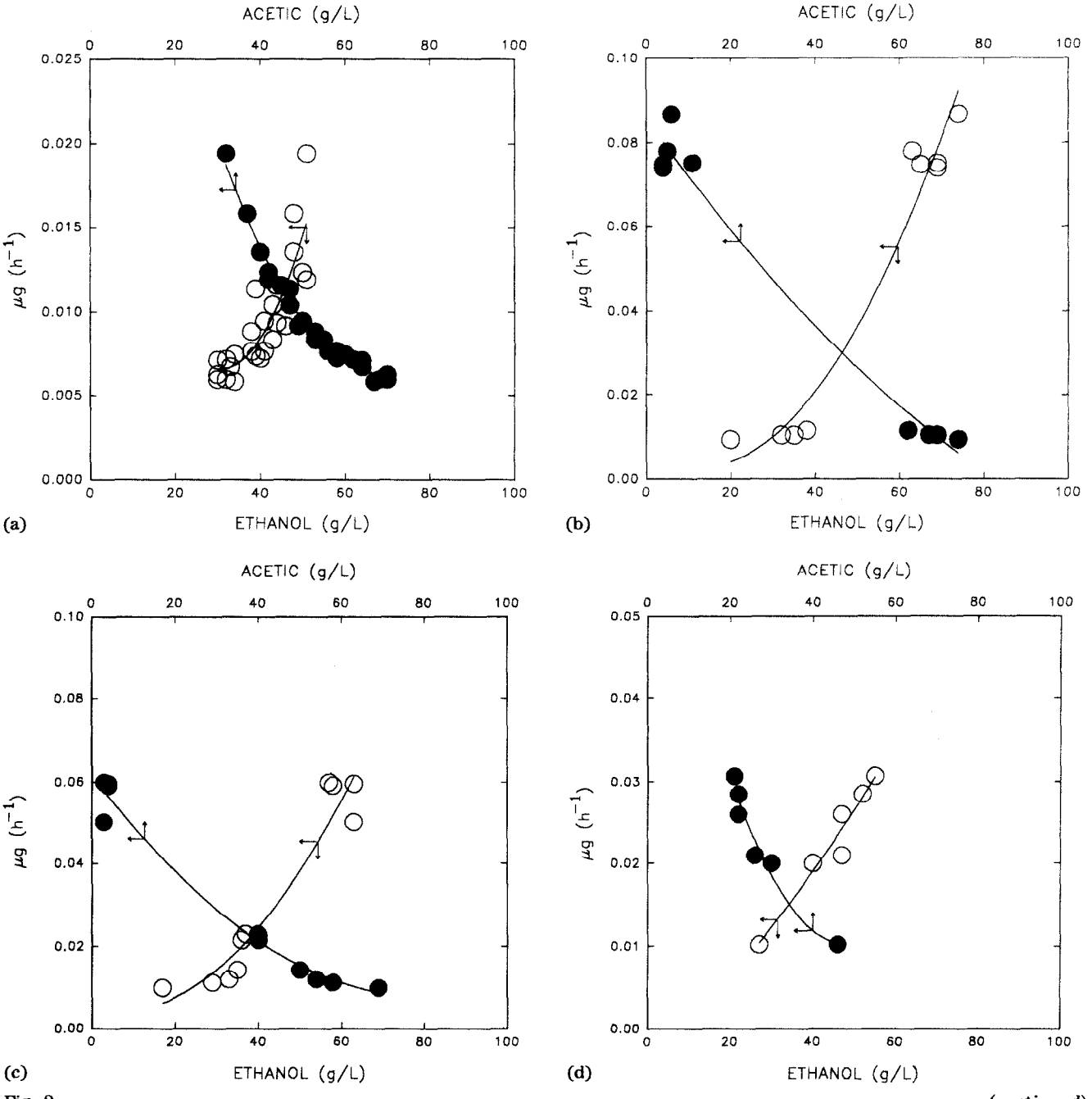


Fig. 3 *(continued)*

Firstly, the controlling enzyme concentration must be constant in the viable cells, and secondly, there must be no significant inward cellular diffusional limitations on the part of the compounds included in the growth equation. These approximations are both reasonable if its is assumed that the viable cells have a standard enzymatic charge and good capacity for transportation of the nutrients and metabolites through their membranes.

In short, bearing in mind the above mentioned considerations, the specific cellular growth rate may be directly related to the rate of the enzymatic reaction controlling the energy metabolism. Therefore, on the basis of these considerations, the proposed kinetic growth equation (eqn. (6)) may respond to the enzymatic action mechanism in the reaction.

Because enzymatic action mechanisms are generally quite complex, it is difficult to deduce precisely the kinetic pattern of the microbial growth controlling step on the basis of the experimental work developed in this study. However, Fig. 4 is included

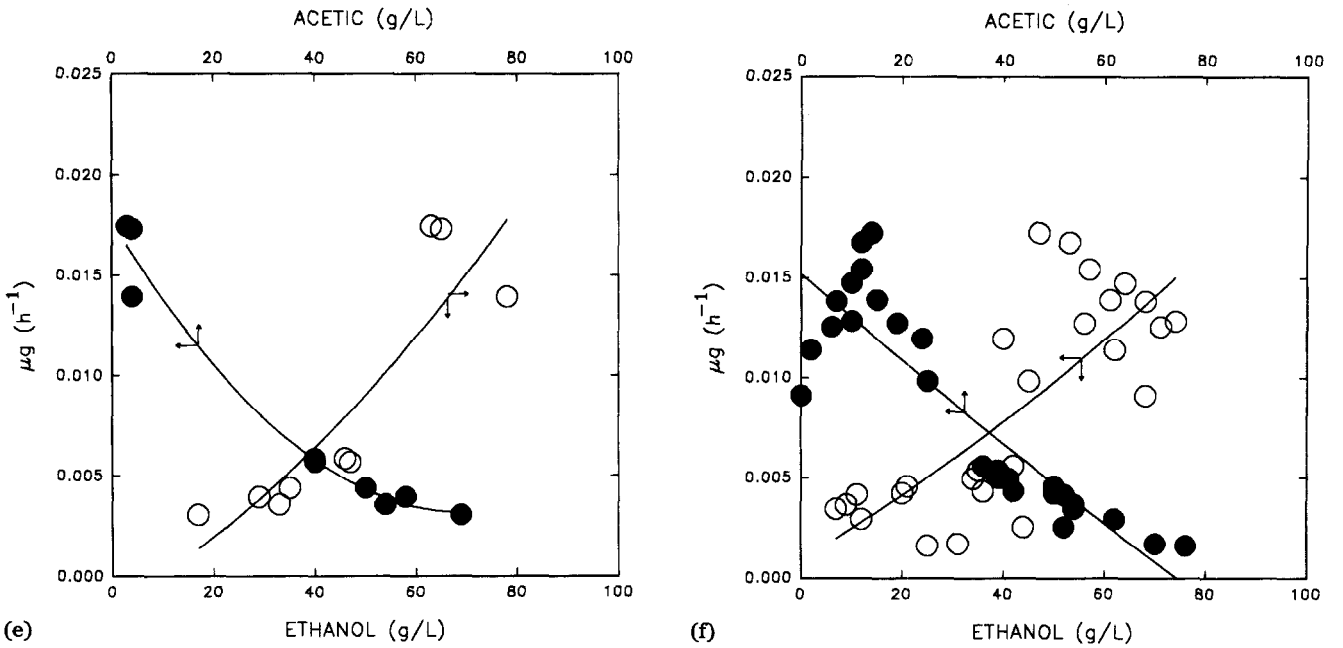


Fig. 3. Set of experimental data on overall specific growth rate as a function of the three variables examined (ethanol, acetic acid and oxygen). (a) 0.5 p.p.m. oxygen; (b) 1.0 p.p.m. oxygen; (c) 3.0 p.p.m. oxygen; (d) 4.5 p.p.m. oxygen; (e) 6.0 p.p.m. oxygen; (f) 7.0 p.p.m. oxygen.

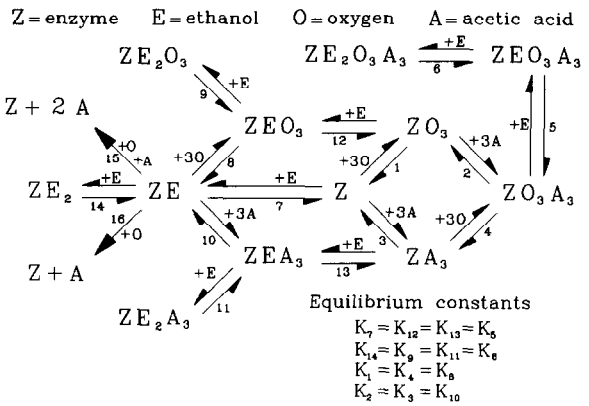


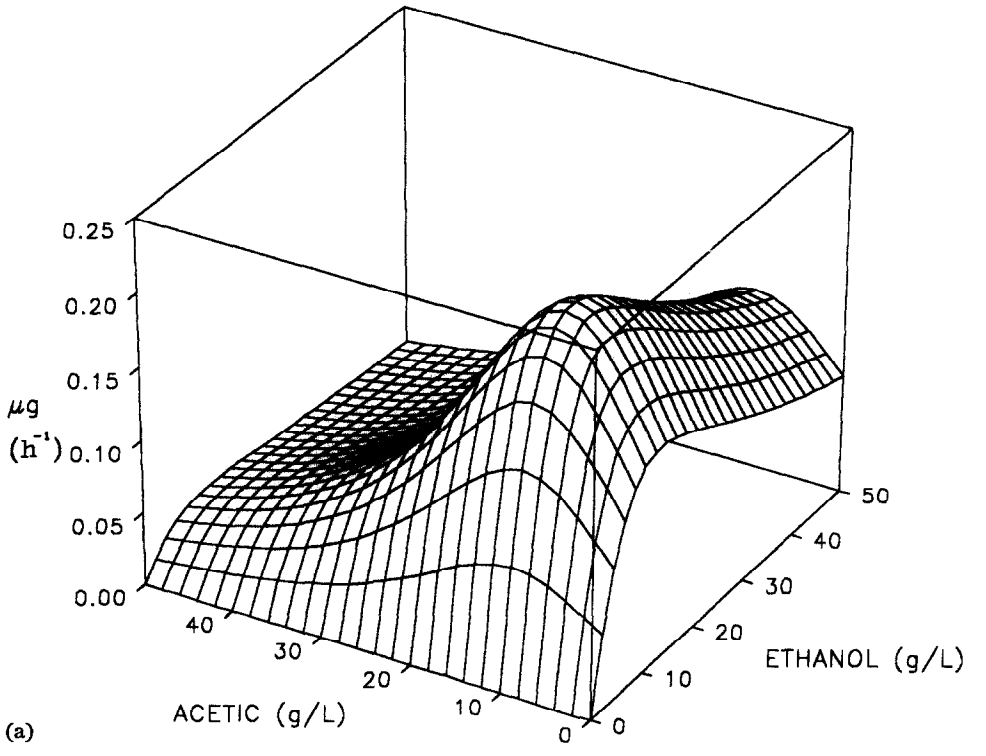
Fig. 4. One of the possible enzymatic mechanisms corresponding to the proposed mathematical growth equation. By applying steady state considerations and the set of special simplifications summarised at the bottom of the figure, eqn. (6) can be obtained.

to show that there might be some potential mechanisms which respond well to the proposed mathematical equation (eqn. (6)). This scheme takes into account a large number of simplifications, but it is a proposal completely in line with the kinetic behaviour generally observed in the growth of *Acetobacter aceti* in submerged cultures.

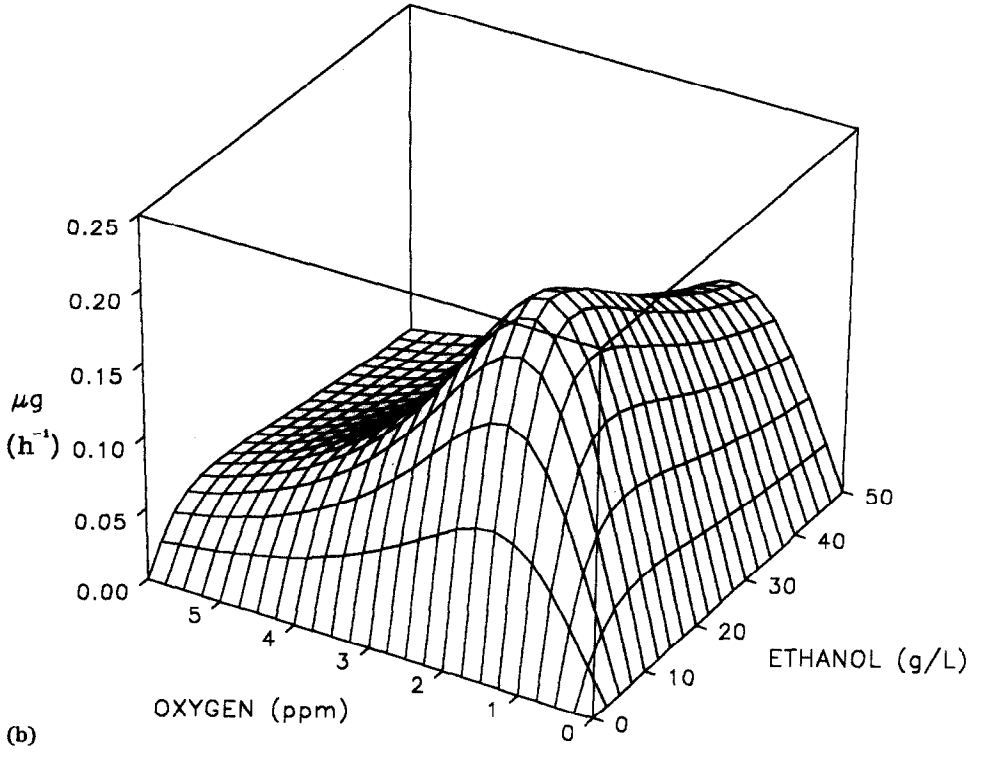
4.6. Values calculated for the coefficients

To obtain the mathematical adjustment of the coefficients in eqn. 6, a non-linear regression procedure was used based on Marquardt's algorithm [18]. Application of this procedure to the set of experimental data in Fig. 3 gave the following values of the parameters. $\mu_{max} = 0.22 \pm 0.02$ h^{-1}, $K_{SE} = 21.1 \pm 6.7$ g L^{-1}, $K_{SA} = 12.6 \pm 2.5$ g L^{-1}, $K_{SO} = 0.372 \pm 0.05$ p.p.m., $K_{IE} = 2.83 \pm 0.2$ g L^{-1}, $K_{IA} = 17.9 \pm 1.2$ g L^{-1}, $K_{IO} = 1.958 \pm 0.21$ p.p.m., $r^2 = 0.97$. The following considerations may be made in relation to this set of adjusted values. In the first place, the value of the coefficient K_{SE} indicates a high metabolic affinity of *Acetobacter aceti* for ethanol (compared with the value $K_{SG} = 112$ g L^{-1} [19] in the case of *Saccharomyces cerevisiae* yeast for glucose in one of the best exploited industrial fermentation systems, alcohol fermentation). Secondly, the value of K_{IE} shows that ethanol markedly inhibits the growth of *Acetobacter aceti*, as would be expected from a compound of general antiseptic nature such as ethanol. Other non-antiseptic substrates usually show a much lower level of inhibition, hence $K_{IG} = 28.8$ g L^{-1} for inhibition of the growth of *Propionibacterium freundeureichii* by glucose [20]. In consequence, on the basis of the parameter values obtained, the optimal ethanol concentration E_{max} for the growth of *Acetobacter aceti* is 12.99 g L^{-1}.

In reference to the product coefficients, the value for K_{SA} shows that acetic acid has a certain enhancing effect on the growth of the bacterium. This effect of acid compounds is not usually noted on microorganisms, except where they are adapted to such conditions. However, the value of the K_{IA} parameter



(a)



(b)

Fig. 5. Graphic representation of the proposed equation (eqn. (6)) for different parameter values calculated from the experimental data. (a) 1.5 p.p.m. oxygen; (b) 10 g L^{-1} acetic acid.

shows that this acid has a significant inhibiting effect on growth. As a reference value, $K_{IE} = 50$ g L^{-1} for the effect of ethanol on the growth of *Saccharomyces cerevisiae* during alcohol fermentation [19]. As a consequence, according to the parameter values obtained, the optimum acetic acid concentration A_{max} for the growth of the bacterium under examination is 9.95 g L^{-1}.

As to the oxygen coefficients, the reduced value of K_{SO} shows the high affinity of this bacterium for oxygen, clear evidence of its nature as a strictly aerobic microorganism. However, the second oxygen coefficient value K_{IO} shows that, in conditions of high aeration, there is a significant inhibition of growth. While this effect might in principle be abnormal, it has commonly been noted in a large number of aerobic microorganisms when there are high oxygen concentrations in the medium. In such conditions, the redox systems in those cells are unbalanced with a resulting generalised loss of control of their metabolic pathways. The optimum dissolved oxygen concentration O_{max} for the growth of *Acetobacter* is 1.55 p.p.m., calculated from the parameter values obtained.

Finally, the value obtained for μ_{max} under the experimental conditions is slightly lower than the values obtained under other conditions [1, 2, 3], demonstrating the high dependence of this parameter upon other operational variables such as temperature or the characteristics of the culture medium. In this sense, it must be pointed out that the presence of growth inhibitors (such as sulphur dioxide) may be decisive for the overall value observed.

Figure 5 shows the dependence of the overall specific growth rate on the three variables studied under the experimental conditions used here. This representation corresponds to the plot of eqn. (6) for the coefficient values set out above.

5. Conclusions

In the first instance it is confirmed that *Acetobacter aceti* growth in alcoholic substrates is affected in general by three types of inhibition, substrate (ethanol), product (acetic acid) and oxygen, when these compounds are present in the fermentation medium in high concentrations. Moreover, it is also confirmed that the main fermentation product does to some degree enhance growth.

These effects are contained with sufficient accuracy in the proposed growth equation (eqn. (6)), whose theoretical basis lies in the mechanisms of enzymatic action. In addition to the theoretical basis referred, the equation is highly useful thanks to its entirely separate consideration of the influences of the different variables on the specific growth rate.

Finally, the parameters introduced into the equation take on a simple physical significance, and their adjusted values enable interesting calculations to be applied in the design and development of industrial acetic fermentation processes. In this sense the estimated optimum concentrations for growth are: ethanol 13 g L^{-1}, acetic acid 10 g L^{-1}, oxygen 1.6 p.p.m.

Acknowledgments

The authors wish to thank the company Pedro Domecq, S.A. for its collaboration in this study, in the supply of raw materials, microorganisms and industrial fermentation equipment, and for its financial support in the form of research grant no. 1I0-100/92.

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Appendix A: Nomenclature

$(\,)_i$	value for the instant i
A	acetic acid concentration (g L^{-1})

E ethanol concentration (g L^{-1})
K_{ij} inhibition constant referred to species j (g L^{-1}, p.p.m.)
K_{sj} saturation constant referred to species j (g L^{-1}, p.p.m.)
O oxygen concentration (p.p.m.)
r^2 non-linear determinating coefficient
t time (h)
t_{i-1} initial instant of a given interval i (h)
t_i final instant of a given interval i (h)
X total biomass concentration (gDW L^{-1})
X_n non-viable biomass concentration (gDW L^{-1})
X_v viable biomass concentration (gDW L^{-1})
Y_i parameter or variable Y at instant i
Y_{max} parameter or variable Y corresponding to μ_{max}

Greek letters

μ observed specific growth rate (h^{-1})
μ_g overall specific growth rate (h^{-1})
μ_{gj} specific growth factor for species j (h^{-1})
μ_d overall specific death rate (h^{-1})
μ_{max} maximum observed specific growth rate (h^{-1})
μ_{maxj} maximum specific growth factor for species j (h^{-1})