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Inhibitory effect of dihydroxyacetone on *Gluconobacter oxydans*: Kinetic aspects and expression by mathematical equations

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SUMMARY

Microbial conversion of glycerol into dihydroxyacetone (DHA) by *Gluconobacter oxydans* was subjected to inhibition by excess substrate. Comparison of cultures containing increasing initial DHA contents (0 to 100 g l^{-1}) demonstrated that DHA also inhibited this fermentation process. The first effect was on bacterial growth (cellular development stopped when DHA concentration reached 67 g l^{-1}), and then on oxidation of glycerol (DHA synthesis only occurred when the DHA concentration in the culture medium was lower than 85 g l^{-1}). Productivity, specific rates and, to a lesser extent, conversion yields decreased as initial concentrations of DHA increased. The changes in the specific parameters according to increasing initial DHA contents were described by general equations. These formulae satisfactorily express the concave aspect of the curves and the reduction in biological activity when the cells were in contact with DHA concentrations of up to 96 g l^{-1}.

INTRODUCTION

The microbial conversion of glycerol results in various metabolites of industrial importance: dihydroxyacetone, 1,3-propanediol [5], hydroxypropionaldehyde [9], etc. Up until today, the only fermentation process to be developed on an industrial level is DHA production. In *Gluconobacter oxydans*, an inhibitory effect linked to glycerol concentration affects the kinetic parameters but not the DHA yields [3]. However, the drop in glycerol-to-DHA conversion yields observed by several authors (e.g., Refs. 10, 11) in cultures of high initial substrate concentration could not be ascribed to a direct effect of glycerol on cellular activity, and suggests other inhibitory factors may be involved.

In our earlier papers [2,3], we postulated an inhibitory effect linked to DHA. We demonstrated that bacterial growth and oxidation of glycerol ceased when the DHA concentration in the medium reached 61 g l^{-1} and 110 g l^{-1}, respectively.

In the literature, there is little information on inhibition by DHA in micro-organisms. Nevertheless, a negative effect of DHA has been reported on bacterial growth [4] and the stability of glycerol dehydrogenase [8], the enzyme responsible for the conversion of glycerol.

The present study concentrated on kinetic characterisation and expression by mathematical equations of DHA-dependent inhibition of glycerol oxidation in *Gluconobacter oxydans*.

MATERIALS AND METHODS

Experimental organism

Gluconobacter oxydans strain ATCC 621 was used. It was maintained on agar slants containing 20 g l^{-1} glycerol, 10 g l^{-1} yeast extract, and 20 g l^{-1} agar, and transferred to new slants every month.

Cultures

Inocula (200 ml) were prepared in 500-ml flasks, and placed on an orbital shaker (130 rpm) in a thermostated room (28 °C) for 1 day. The growth medium containing 50 g l^{-1} glycerol, and 10 g l^{-1} Difco Yeast Extract.

A concentrated DHA solution (200 ml) was added after

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Abbreviations: X, S, P, biomass, substrate, product concentrations; r_x, r_s, r_p, rates of growth, consumption and production; μ, q_s, q_p, specific rates of growth, glycerol consumption and DHA production; $Y_{x/s}$, $Y_{p/s}$, conversion yields of substrate into biomass and product; K_s, constant of affinity of cells to the substrate; K_{ip}, product inhibition constant; P_m, threshold concentration of DHA in substrate.

sterilisation of the medium, since the compound reacts strongly with the yeast extract protein.

The cultures were made up in 2-litre LSL Biolafitte bioreactors initially containing 1.5 litres of growth medium. Inoculation was carried out by adding 100 or 200 ml of precultures.

The pH was adjusted to 6 (addition of 2 M NaOH) and the temperature to 28 °C. Agitation (800 rpm) and aeration (1 air volume/reactor volume/minute (vvm)) ensured that the partial pressure of oxygen remained at a level greater than 10% saturation during all experiments. Foam formation was prevented by adding Sigma Silicone Emulsion diluted to one-tenth.

Analytical methods

Estimation of biomass concentration. Optical density (OD) was measured at 620 nm with a Varian spectrophotometer. Centrifuged samples (20000 rpm, 5 °C, 10 min) were used as controls and for dilutions. It was found that one OD unit corresponded to 450 mg l^{-1} dry weight.

To determine dry weight of the organisms, 40-ml samples were taken from the culture, centrifuged, washed twice with distilled water and dried to constant weight at 105 °C.

Determination of metabolites. Glycerol and DHA contents were determined by HPLC after filtration and dilution of samples, using a Brownlee Polypore Calcium column thermostated at 86 °C and eluted by a continuous flow (0.4 ml min^{-1} with a Shimadzu L6A pump) of bi-distilled, filtered and de-aerated water. Compounds were detected with a Waters refractometer and their concentrations evaluated using a Shimadzu Integrator CR 3A.

RESULTS

Inhibitory effect of DHA

The effect of DHA on glycerol conversion by *Gluconobacter oxydans* was analysed by comparing cultures initially containing 50 g l^{-1} substrate and increasing concentrations of DHA (from 0 to 100 g l^{-1}).

The first inoculation ratio to be tested was 5.9% v/v (the same as that used to study the inhibitory effect of glycerol). Under these conditions, bacterial growth and glycerol oxidation occurred only when the initial DHA content was lower than 30 g l^{-1}. DHA had a very pronounced inhibitory effect on cellular activity since a level of 30 g l^{-1} at the beginning of culture (inoculated at 5.9% v/v) was lethal to *G. oxydans*. The inoculation ratio was then increased to 10.8% v/v. At this level, the critical initial DHA concentration for biological activity was about 100 g l^{-1}. These same experimental conditions were used for studying DHA-dependent inhibition of glycerol oxidation in *G. oxydans*.

The integral (X, S, P) and specific (μ, q_s, q_p) parameters were determined from cultures of varying DHA content (0, 13.9, 26, 37.6, 47.7, 68.7 and 100 g l^{-1}) and identical glycerol concentration, i.e., 50 g l^{-1} (Figs. 1 and 2). In general, the presence of DHA at the beginning of the culture resulted in increased duration of fermentation: the time required by the micro-organisms to oxidise 47.7 g l^{-1} glycerol was 3-times greater for the culture containing 47.7 g l^{-1} DHA than the control (i.e., without initial addition of DHA).

In cultures where the initial DHA content was lower than 47.7 g l^{-1}, the 50 g l^{-1} glycerol was consumed entirely. On the other hand, when the culture started off at 68.7 g l^{-1} DHA, only 19.5 g l^{-1} glycerol were consumed after 60 h. Comparison of the characteristic parameters of each experiment (Table 1) showed that the increased duration of fermentation was linked to an inhibitory effect of DHA on the kinetics of bacterial growth and glycerol oxidation, and on biomass and DHA production.

Inhibition of production kinetics. The effect of DHA on bacterial conversion of glycerol became first apparent on the level of kinetics. When fermentation occurred at an initial DHA content of 26 g l^{-1}, maximum productivities and specific rates represented 57% and 70% of the corresponding parameters obtained in the control culture (i.e., no initial DHA). When the initial DHA level was 68.7 g l^{-1}, these parameters reached a maximum of 4% and 20% of these same reference values. A similar decrease was observed for all parameters studied: specific growth rate, specific rate of glycerol consumption, and specific rate of DHA production (Table 1). The kinetic parameters reached their optimum: level at an early stage of the culture (between the 3rd and 5th h for specific growth rate, and between the 5th and 15th for specific rates for glycerol consumption and DHA production). Thenceforward, their values continued to decrease at varying speeds according to initial DHA concentration (Fig. 2).

These results demonstrated that low initial DHA contents (< 13 g l^{-1}) restricted cellular activity right from the beginning of fermentation. Increasing initial DHA concentrations (supplied or produced) accentuated this.

Effects of DHA on bacterial growth. For an identical quantity of glycerol consumed (50 g l^{-1}), as initial DHA content increased, the concentration of synthesised biomass decreased; this was expressed by decreased conversion of substrate into biomass (Table 1).

Concerning cultures of initial DHA content greater than 26 g l^{-1}, a simultaneous analysis of DHA production and biomass synthesis (Fig. 1), revealed that growth stopped when the DHA concentration in the cultured medium reached a threshold value of about 67 g l^{-1}.

Under these conditions, unlike the control, although a certain amount of glycerol remained in the culture medium and DHA production continued, microbial growth ceased.

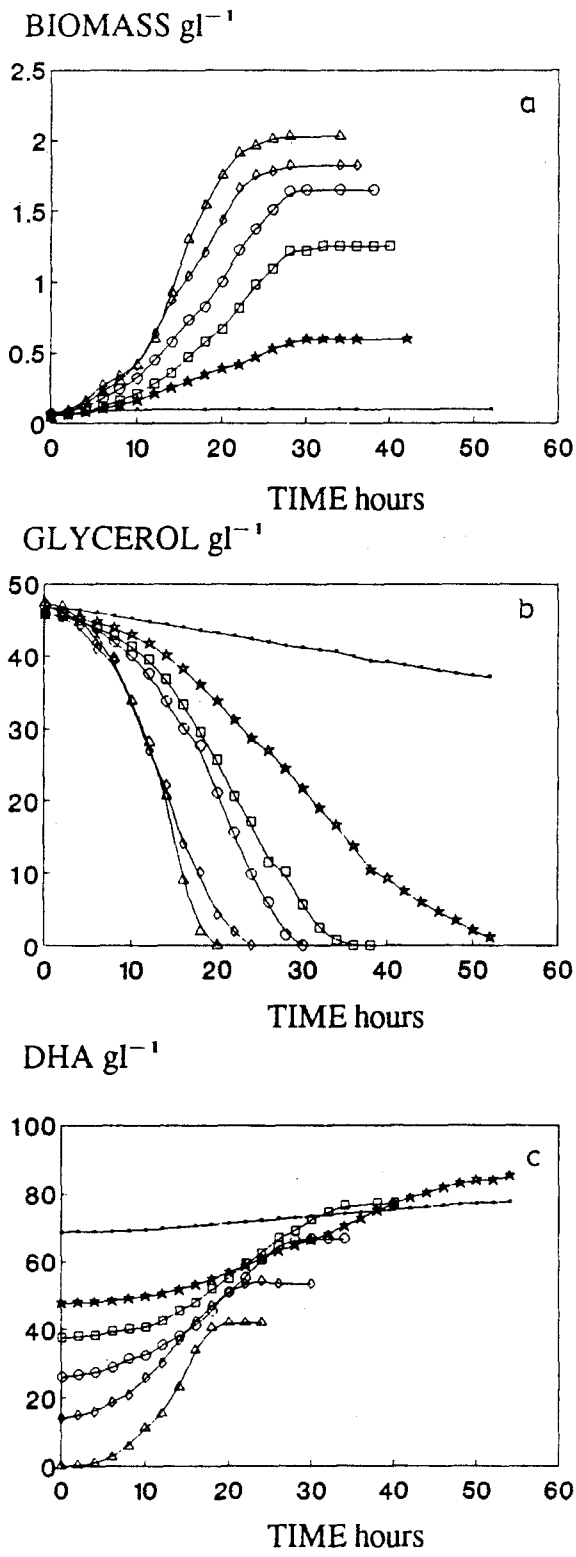


Fig. 1. Changes in the biomass (a), glycerol (b) and DHA (c) concentrations in cultures of *Gluconobacter oxydans* on glycerol 50 g l^{-1} performed with increasing initial DHA concentrations. 0 (△), 13.9 (◇), 26 (○), 37.6 (▽), 47.7 (□), 68.7 (★) and 100 (●) g l^{-1} DHA.

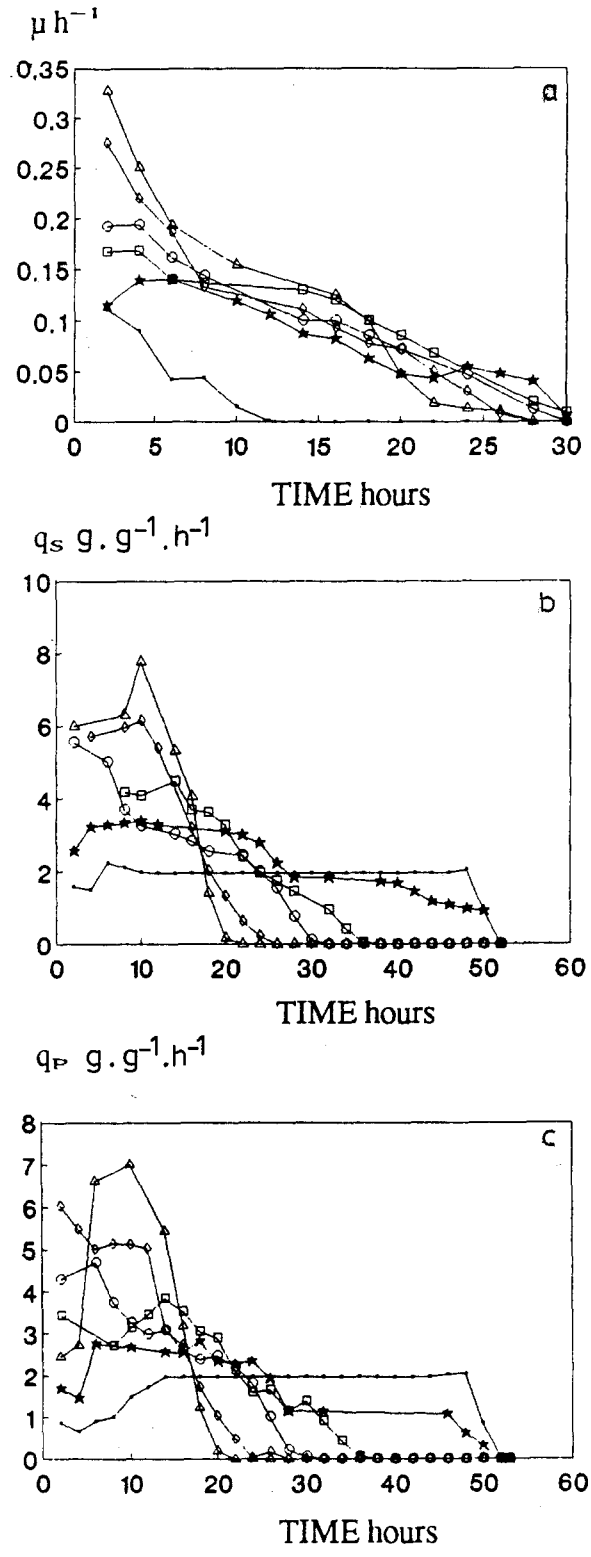


Fig. 2. Changes in the specific growth rates (a), the specific rates of glycerol consumption (b) and the specific rates of DHA formation (c) in cultures of *Gluconobacter oxydans* performed with increasing initial DHA concentrations. 0 (△), 13.9 (◇), 26 (○), 37.6 (▽), 47.7 (□), 68.7 (★) and 100 (●) g l^{-1} DHA.

TABLE 1

Determination of characteristic parameters of *G. oxydans* cultures performed with increasing levels of initial DHA content

Initial DHA content ($g\,l^{-1}$)	0	13.9	26	37.5	47.7	68.7	100
Duration (h)	20	24	30	36	55	60	–
Biomass producted ($g\,l^{-1}$)	2.0	1.8	1.6	1.2	0.6	0.1	0.06
Glycerol consumed ($g\,l^{-1}$)	47.4	46	46.9	45.8	45.8	19.5	0
DHA produced ($g\,l^{-1}$)	41.9	39.3	40.6	39.4	36.2	8.7	0
$Y_{x/s}$ (%)	4.3	4.0	3.5	2.7	1.3	0.5	–
$Y_{p/s}$ (%)	88.4	85.4	86.6	86.0	79.0	47.7	0
r_x ($g\,l^{-1}\,h^{-1}$)	0.16	0.11	0.09	0.08	0.02	0.01	–
r_s ($g\,l^{-1}\,h^{-1}$)	5.08	3.44	2.94	2.29	1.53	0.02	–
r_p ($g\,l^{-1}\,h^{-1}$)	5.03	3.23	2.75	2.09	1.26	0.20	–
μ ($g\,l^{-1}\,h^{-1}$)	0.33	0.27	0.22	0.17	0.14	0.06	–
q_s ($g\,l^{-1}\,h^{-1}$)	7.78	6.65	5.74	4.59	3.43	1.56	–
q_p ($g\,l^{-1}\,h^{-1}$)	7.00	5.82	4.69	3.89	2.79	1.40	–

These two biological activities (cellular development and DHA formation) were thus distinct. This suggested that DHA had a selective effect on the biological system's dynamic behaviour: synthesis of biomass was sensitive to DHA concentrations (67 $g\,l^{-1}$) lower than those at which oxidation of glycerol ceased (85 $g\,l^{-1}$). This could explain the decrease of biomass production under increasing DHA concentrations.

Effect of DHA on DHA production. Conversion of substrate into DHA was also affected by the presence of DHA, although to a lesser extent (Table 1, Fig. 3). It was constant for initial DHA concentrations of 0 to 37 $g\,l^{-1}$, then decreased substantially as concentration increased (Fig. 3). Here, the reduction in yield may be ascribed to increased exploitation of the substrate by the cells to ensure the production of energy, as well as survival in an increasingly hostile environment.

There was a distinct difference between the threshold concentration at which conversion of substrate into biomass was affected ($< 13.9\ g\,l^{-1}$), and that affecting conversion of substrate into DHA (37 $g\,l^{-1}$). Given the greater effect on growth than on DHA synthesis, this phenomenon would seem to confirm the selective effect of DHA.

Expression by mathematical equations of DHA inhibition of the kinetics of bacterial growth and DHA production

Most of the models expressing DHA's inhibitory effects listed in the references [6,7] were tested for purposes of modelisation (Tables 2 and 3). Given the non-limiting glycerol concentration (50 $g\,l^{-1}$) used in the present study, the low value of constant K_s, (affinity of cells to the substrate), assessed at 4 $g\,l^{-1}$ [3] enabled the term $S/(K_s + S)$ to be omitted.

Inverse and exponential models. The model of Bazua et al. must be considered as different to other formulae since its constants k and b have no biological signification. The correlation coefficients, expressing the difference between the experimental data and the theoretical curves obtained by this model, demonstrated that the equation did not fit the phenomenon observed especially in the case of DHA production (Figs. 4 and 5).

Comparison of the theoretical curves obtained by the inverse (Egamberdiev) or exponential (Aïba) models and the experimental data showed that these formulae only described the experiments satisfactorily for DHA concen-

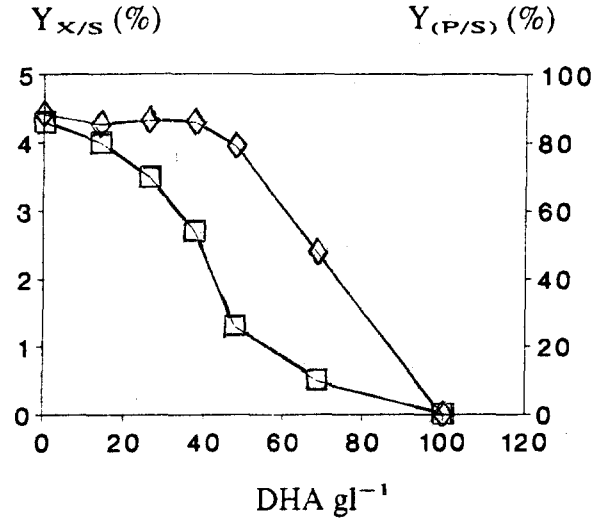


Fig. 3. Effect of initial DHA concentration on conversion yields of substrate into biomass (□) and product (◇) by *Gluconobacter oxydans*.

TABLE 2

Modelisation of the inhibitory effect of DHA on bacterial growth

Model equation	Simplified expression	Constant calculation	Correlation coefficient
Ghose et al.'s model $\mu = \mu^{max} \cdot \frac{S}{S + K_s} \cdot (1 - P/P_m)$	$\mu = \mu^{max} \cdot (1 - P/P_m)$	$P_m = 86.0$ $\mu^{max} = 0.328$	0.992
Egamberdiev's model $\mu = \mu^{max} \cdot \frac{S}{S + K_s} \cdot \frac{K_{ip}}{P + K_{ip}}$	$\mu = \mu^{max} \cdot \frac{K_{ip}}{P + K_{ip}}$	$K_{ip} = 72.12$ $\mu^{max} = 0.328$	0.966
Bazua's model $\mu = \mu^{max} \cdot \frac{S}{S + K_s} \cdot (1 - k \cdot P/(b - P))$	$\mu = \mu^{max} \cdot (1 - k \cdot P/(b - P))$	$k = 3.01$ $b = 272.7$	0.992
Aïba's model $\mu = \mu^{max} \cdot \frac{S}{S + K_s} \cdot \exp(-P/K_{ip})$	$\mu = \mu^{max} \cdot \exp(-P/K_{ip})$	$K_{ip} = 78.4$ $\mu^{max} = 0.328$	0.989
Luong's model $\mu = \mu^{max} \cdot \frac{S}{S + K_s} \cdot (1 - P/P_m)$	$\mu = \mu^{max} \cdot (1 - (P/P_m)^{\alpha})$	$\mu^{max} = 0.328$ $P_m = 83.9$ $\alpha = 0.984$	0.995
Levenspiel's model $\mu = \mu^{max} \cdot \frac{S}{S + K_s} \cdot (1 - P/P_m)$	$\mu = \mu^{max} \cdot (1 - (P/P_m))^{\alpha}$	$\mu^{max} = 0.328$ $P_m = 87.0$ $\alpha = 1.09$	0.997

Calculation of the characteristic constants given in several models proposed in the literature. Application to experimental results.

trations between 0 and 26 g l^{-1} (Figs. 4 and 5). Above this upper value, the curves deviate from the experimental data. Similarly, the values of K_{ip} (the product inhibition constant) determined for both biological activities investigated (bacterial growth and DHA production) by the inverse or exponential models were between 70 and 80 g l^{-1}. Consequently, these results disagreed with the experimental data because, (a) the constant K_{ip} theoretically represents the product concentration for which specific rates were 2- or 2.7-times lower (inverse and exponential models, respectively) than the maximum specific rate (obtained in the absence of the inhibitory product) and (b) because between 70 and 80 g l^{-1}, the biological activity experimentally observed was practically zero. The inverse and exponential mathematical equations were thus unsuitable for modelling growth inhibition and glycerol conversion by *G. oxydans*.

Linear and general models. Linear (Ghose et al.) and general (Levenspiel, Luong) models [7] involve the use of the threshold concentration of DHA, P_m (beyond which no biological activity occurred), coefficient (expressing the curvature of the curves $\mu = f(P)$ and $q_p = f(P)$ ($x = 1$ in the linear model)), and the maximum specific parameter determined in the absence of inhibitory product (μ^{max}, q_p^{max}).

By means of the constant, P_m, linear or general formulae take into account the absence of microbial activity beyond a threshold of initial DHA concentration. They thus described the phenomenon satisfactorily; this was corroborated by the agreement between theoretical curves and experimental results (Figs. 4 and 5).

The satisfactory correlation coefficient indicated that the general models fitted the experimental results well. However, comparing the model's curves with those of the experiment démonstrated that, at high initial DHA contents, the model of Levenspiel described the decrease of specific parameters best, especially the specific rate of DHA production, q_p (Figs. 4 and 5).

The equations selected to express DHA's dual inhibitory effect on bacterial growth and glycerol oxidation were:

TABLE 3

Modelisation of the inhibitory effect of DHA on DHA formation

Model equation	Simplified expression	Constant calculation	Correlation coefficient
Ghose et al.'s model $q_p = q_p^{max} \cdot \frac{S}{S + K_s} \cdot (1 - P/P_m)$	$q_p = q_p^{max} \cdot (1 - P/P_m)$	$P_m = 82.4$ $q_p^{max} = 0.328$	0.988
Egamberdiev's model $q_p = q_p^{max} \cdot \frac{S}{S + K_s} \cdot \frac{K_{ip}}{P + K_{ip}}$	$q_p = q_p^{max} \cdot \frac{K_{ip}}{P + K_{ip}}$	$K_{ip} = 68.5$ $q_p^{max} = 7$	0.971
Bazua's model $q_p = q_p^{max} \cdot \frac{S}{S + K_s} \cdot (1 - k \cdot P/(b - P))$	$q_p = q_p^{max} \cdot (1 - k \cdot P/(b - P))$	$k = 3.29$ $b = 285.7$	0.936
Aïba's model $q_p = q_p^{max} \cdot \frac{S}{S + K_s} \cdot \exp(-P/K_{ip})$	$q_p = q_p^{max} \cdot \exp(-P/K_{ip})$	$K_{ip} = 75.3$ $q_p^{max} = 7$	0.996
Luong's model $q_p = q_p^{max} \cdot \frac{S}{S + K_s} \cdot (1 - (P/P_m))$	$q_p = q_p^{max} \cdot (1 - (P/P_m)^{\alpha})$	$q_p^{max} = 7$ $P_m = 87.1$ $\alpha = 0.9465$	0.997
Levenspiel's model $q_p = q_p^{max} \cdot \frac{S}{S + K_s} \cdot (1 - (P/P_m))$	$q_p = q_p^{max} \cdot (1 - (P/P_m))^{\alpha}$	$q_p^{max} = 7$ $P_m = 96.0$ $\alpha = 1.205$	0.998

Calculation of the characteristic constants given in several models proposed in the literature. Application to experimental results.

$\mu = 0.378\,(1 - (P/87.1))^{1.095}$
$q_p = 7(1 - (P/96.0))^{1.205}$

The values of coefficients, expressing change of slope in curves $\mu = f(P)$ and $q_p = f(P)$, were greater than 1 (Tables 2 and 3). Hence, within a range of low product concentrations, an increase in DHA content resulted in a greater decrease in specific parameters than within a range of high DHA contents (concave shape of the curves).

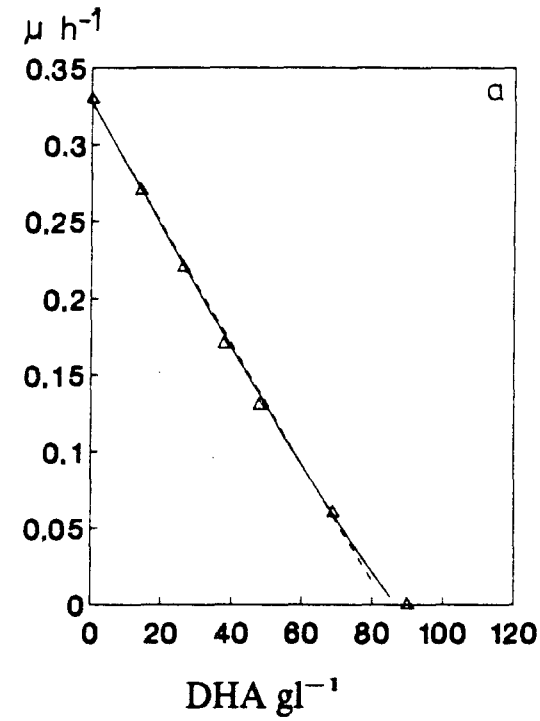
DISCUSSION AND CONCLUSION

The inhibitory effect of DHA on glycerol conversion occurring a different levels of cellular activity in *G. oxydans* was determined and quantified.

In terms of the dynamic behaviour of cultures, we demonstrated that bacterial growth and DHA production were stopped when the DHA concentration in the medium reached 67 and 85 g l^{-1}, respectively. DHA acted selectively on the culture's dynamics, and had a greater effect on bacterial growth than glycerol conversion. Similarly, overall yields of DHA production were affected by initial DHA concentrations lower (< 13.9 g l^{-1}) than that at which the yield of glycerol conversion into DHA decreased (37 g l^{-1}).

The effect of DHA on the kinetics of bacterial growth and glycerol oxidation became apparent by changes in the maximum values of the kinetics parameters (a drop of 80% for the specific rate of DHA production and 82% for the specific growth rate when the DHA concentration rose from 0 to 68.7 g l^{-1}) and in their variation with time in the cultures.

The specific parameters (μ, q_s, q_p) reached their optimum level at the start of culture; following this, their values decreased in a continuous manner. The cells were thus unable to express their growth and glycerol-oxidation potentials in the presence of DHA. We therefore propose the following mode of action for DHA on the bacteria's enzyme system: DHA binds directly and irreversibly to the active sites of enzymes involved in growth and of glycerol dehydrogenase (responsible for oxidising glycerol into DHA) and/or blocks the synthesis of these proteins. The



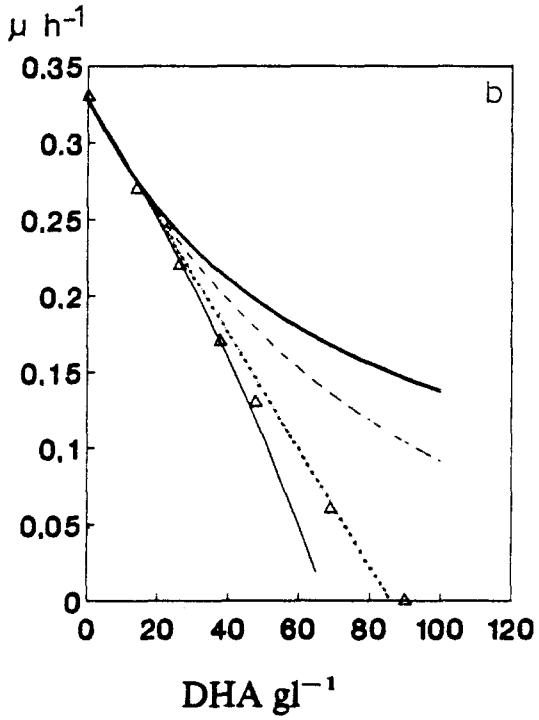
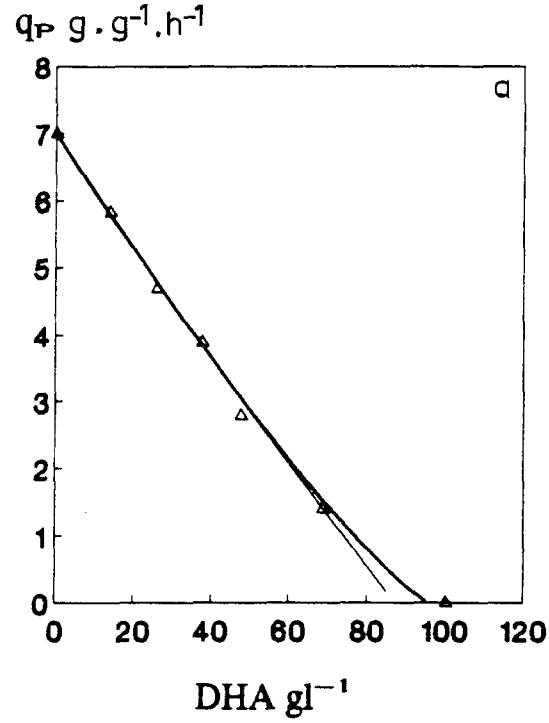


Fig. 4. Modelisation of changes in the specific growth rates with increasing initial DHA contents. Comparison of the models proposed in the literature and the experimental data. (a) (△) Experimental data; (—) Luong's equation; (—) Levenspiel's equation. (b) (△)Experimental results; (...) Linear equation; (—) Inverse equation; (—) Bazua's equation; (– –) Exponential equation.



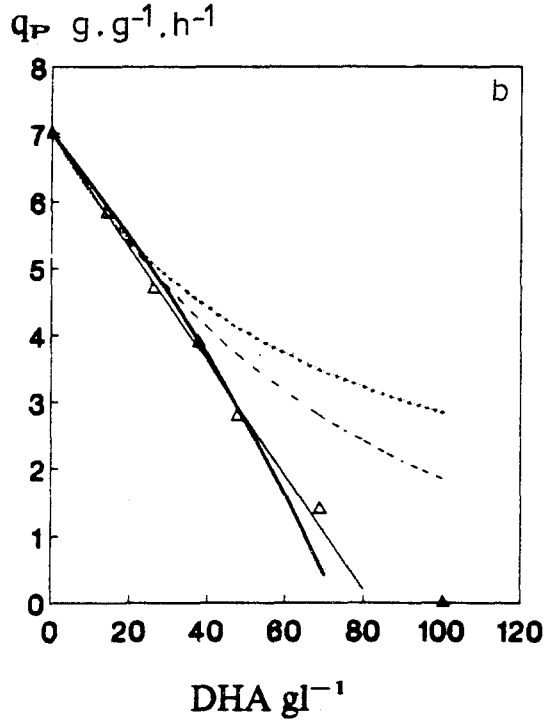


Fig. 5. Modelisation of changes in the specific rates of DHA production with increasing initial DHA contents. Comparison of the models proposed in the literature and the experimental data. (a) (△) Experimental data; (—) Luong's equation; (—) Levenspiel's equation. (b) (△)Experimental results; (—) Linear equation; (—) Bazua's equation; (...) Inverse equation; (– –) Exponential equation.

work of Nabe et al. [8], demonstrating the reduced stability of glycerol dehydrogenase in the presence of DHA, and the well-known reactivity of DHA with the amines of proteins (formation of Schiff's base with glycin), would confirm this hypothesis.

These formulations enabled the experimental phenomenon to be quantified. We have decided to use the general model of Levenspiel: $\mu = 0.328(1 - (P/87.1))^{1.095}$ and $q_p = 7(1 - (P/96))^{1.205}$.

Unlike inverse or exponential equations where curves tend asymptotically towards the x-axis (O,P), this model takes into account the lack of biological activity beyond a threshold DHA concentration. It also expresses, by the x coefficient, the non-linearity of the curves $\mu = f(P)$ and $q_p = f(P)$ which correspond, from a biological point of view, to a difference in cell sensitivity to addition of DHA.

The values of the characteristic constants obtained in modelling the inhibitory effect of DHA on bacterial growth were different from those quantifying the inhibition of DHA production. The initial DHA threshold concentration for bacterial growth ($P_m = 87$ g l^{-1}) was lower than that which blocked the oxidation of glycerol ($P_m = 96$ g l^{-1}). This result confirmed that bacterial growth was more sensitive than was the DHA production system to the presence of DHA.

Inhibition of glycerol oxidation by DHA in *G. oxydans* must be considered differently from inhibition of the same fermentation by the substrate. On the one hand, the consequences of inhibition of conversion by glycerol were far less great than those due to DHA. The reduction in the kinetics of bacterial growth and DHA synthesis linked to addition of DHA was far greater than that due to addition of the same quantity of glycerol. On the other hand, the selective effect of DHA on the various microbial activities was the opposite of the overall action of glycerol on bacterial growth and DHA formation. This may be ascribed to different modes of inhibition in these two compounds. As concerns this, we would suggest that glycerol acts on the cells (at the transport level) in an overall manner, and that DHA is involved to a greater or lesser extent with the enzymes involved in bacterial growth and oxidation of glycerol.

The kinetic study of the dual inhibition of DHA production by both the substrate and the product has increased the understanding of this widely used industrial fermentation process. For example, several hitherto unexplained problems encountered in cultures, such as reduction of yield and productivity with increased initial substrate content, have been attributed to the negative effects of glycerol and DHA on cellular activity, and solved by new fermentation procedures enabling these phenomena to be diminished and production optimised: i.e., the biphasic and fed-batch processes [2].

REFERENCES

1 Blazejak, S. and E. Sobjack. 1988. Bioconversion of glycerol into dihydroxyacetone DHA using *Acetobacter xylinum*. Acta Aliment. Pol. 14: 207–216.

2 Bories, A., C. Claret and P. Soucaille. 1991. Kinetic study and optimisation of the production of dihydroxyacetone from glycerol using *Gluconobacter oxydans*. Proc. Biochem. 26: 243–248.

3 Claret, C., A. Bories and P. Soucaille. 1992. Glycerol inhibition of growth and dihydroxyacetone production by *Gluconobacter oxydans*. Curr. Microbiol. 25: 149–155.

4 Izuo, N., K. Nabe, S. Yamada and I. Chibata. 1980. Production of dihydroxyacetone by continuous cultivation of *Acetobacter suboxydans*. J. Ferment. Technol. 58: 221–226.

5 Forsberg, C.. 1987. Production of 1,3 propanediol from glycerol by *Clostridium acetobutylicum* and other *Clostridium* species. Appl. Environ. Microbiol. 53: 639–643.

6 Luong, J.M.Y. 1987. Kinetics of ethanol inhibition in alcohol fermentation. Biotechnol. Bioeng. 29: 242–248.

7 Mulchandani, A. and J.M.T. Luong. 1989. Microbial inhibition revisited. Enzyme Microbiol. Technol. 11: 66–73.

8 Nabe, K., N. Izuo and I. Chibata. 1979. Conversion of glycerol to dihydroxyatetone by immobilized whole cells of *Acetobacter xylinum*. Appl. Environ. Microbiol. 38: 1056–1060.

9 Slininger, P.J. and R.J. Bothast. 1985. Optimizing aerobic conversion of glycerol to 3-hydroxypropionaldehyde. Appl. Environ. Microbiol. 50: 144–1450.

10 Su, Y.C., K. Koo and S.L. Yin. 1989. Studies on the production of 1,3 dehydroxypropanone by *Gluconobacter oxydans* CCRC 10684. J. Chin. Agric. Chem. Soc. 27: 307–321.

11 Yamada, S., K. Nabe, N. Izuo, M. Wada and I. Chibata. 1979. Fermentative production of dihydroxyacetone by *Acetobacter suboxydans* ATCC 621. J. Ferment. Technol. 57: 215–220.