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INHIBITORY EFFECTS OF DIHYDROXYACETONE ON *GLUCONOBACTER* CULTURES

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Summary

The inhibitory effects of DHA on *Gluconobacter oxydans* were measured to forrmulate a fermentation model. Growth of *Gluconobacter oxydans* is inhibited by DHA which can be modelled by a linear Term. The inhibition of product formation by DHA was measured and described by a classical feedback inhibition kinetic. Additionally, an irreversible destruction of Gluconobacter cells by DHA was discovered. This toxic effect of DHA could be modelled by a death rate kinetic and introduction of a damaged cell type. DHA also inhibits the activity of the pentose cycle as can be measured via the CO_2 evolution rate.

Introduction

Gluconobacter oxydans oxidizes many polyols to ketones by membrane-bound dehydrogenases. Two ketones are of great industrial importance: sorbose, as a precursor for vitamin C synthesis, and dihydroxyacetone (DHA) as a tanning agent for cosmetic purposes. The oxidation products are accumulated in the medium but may be re-assimilated and metabolized further via the pentose phosphate pathway (Asai, 1968). Thus, the production rate is a sum of product formation and product depletion. Both parts may be measured on-line and separately by exhaust gas analysis: the product formation is represented by the O_2 uptake rate, the product metabolism is represented by the CO_2 evolution rate.

Glycerol	+	$^1/_2\ O_2$	--->	DHA +	H_2O	(1)
DHA	+	$3\ O_2$	--->	$3\ CO_2$ +	$3\ H_2O$	(2)

To formulate a fermentation model, we examined the kinetics of glycerol oxidation by *Gluconobacter oxydans*. Although both glycerol and dihydroxyacetone inhibit the fermentation process (Sattler 1964), it is not fully understood how the inhibitions are brought about as the kinetics are not described in detail. Recently, Claret et al. (1994) attempted to clarify the mechanism of DHA inhibition of growth and product formation but they did not take into account product metabolism which occurs as shown by other authors (Tachiki et al. 1987).

Experimental Methods

For kinetic measurements an industrial strain of *Gluconobacter oxydans*, supplied by E. Merck, Darmstadt, was precultured with sorbitol (5 % w/v)- containing medium with 5 g/L yeast extract added as a nitrogen source (T = 30 °C).
For fermentations, pH and pO_2 were controlled (at pH 4,5 and 70 % saturation) using a 5 L-laboratory fermenter (Bioengineering L 1523).The respiratory activity was measured by exhaust gas analysis by mass spectrometry (Perkin Elmer MGA 1200) and on-line data processing. Measurements of O_2 consumption rate and CO_2 formation rate were made in fed batch cultures with glycerol constantly at of 1 % (w/v) and a variable DHA concentration.
The influence of DHA on bacterial growth was examined in shaking flask cultures with a sorbitol-containing medium (200 mL in 1 L-Erlenmeyer flasks) and a variable DHA content. To prevent oxygen limitation the growth rate was determined after only 2 h of incubation (T = 28 °C).
Death rate kinetics were determined similarily. *Gluconobacter* was kept in a fermenter with constant DHA concentration and sorbitol as an energy source. Samples of biomass were taken hourly and used as an inoculum for a sorbitol-containing, DHA-free shake-flask. The observed growth rate in this shake-flask, related to its value at the beginning of the experiment, represents the relative viability of the cells.

Results and Discussion

The inhibition of DHA on growth, product formation, and CO_2 formation of *Gluconobacter oxydans* is shown Fig. 1.

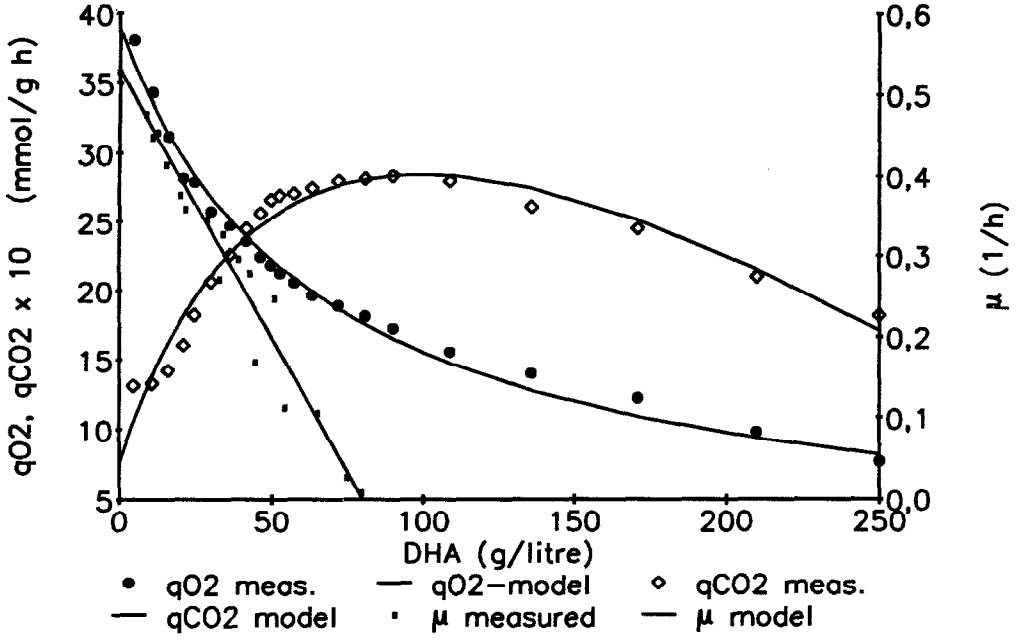

Fig. 1: Inhibition of growth, specific O_2 consumption, and CO_2 evolution of *Gluconobacter oxydans* by dihydroxyacetone

The almost linear kinetics observed resemble the inhibition kinetics found for ethanol with yeast. It can be described by a product inhibition term proposed by Levenspiel (1980):

$$\mu = \mu_m \cdot (1 - P/P_m)^n \qquad n = 1 \qquad (3)$$

Such kinetics are appropriately applied, if there is no growth above a maximum inhibitor concentration, P_m. With the examined strain, this was found to be 80 g DHA/L.

The O_2 uptake rate (qO_2) is directly proportional to the glycerol oxidation rate and therefore, to the product formation rate. Thus, Fig. 1 clearly shows the inhibition of product formation by DHA.

The observed retardation of glycerol oxidation by DHA can be best described by classical feedback inhibition kinetics:

$$qO_2 = qO_{2m} \cdot K_p / (P + K_p). \quad (4)$$

The inhibition of product formation by DHA agrees with the observations of other authors (Sattler 1964, Bories et al. 1991).

According to the process stoichiometry, product concentration is also determined by DHA metabolism, which can be monitored by the CO_2 evolution rate, qCO_2 (Fig. 1). Comparisons of HPLC analysis of glycerol and DHA with calculations based on-off gas analysis showed good agreement and proved the validity of the stoichiometry assumed (eq. 1 & 2).

DHA can be metabolized via the pentose phosphate pathway. Therefore, it is not surprising to find Monod kinetics for the CO_2 evolution up to 5 % DHA but at higher contents of dihydroxyacetone, a pronounced inhibition is observed. This behaviour can be best described by a combination of a Monod-term with a linear inhibition term

$$qCO_2 = qCO_{2m} \cdot P / (P + K_{pc}) \cdot (1-P/P_m) + qCO_{2s} \quad (5)$$

The constant term, qCO_{2s}, accounts for a CO_2 evolution from the complex nutrient or from the direct metabolism of glycerol. Little CO_2 formation can be observed in the absence of DHA.

The above shows how inhibitory effects of DHA on *Gluconobacter oxydans* can describe the equilibrium states, but does not provide any information about the time dependancy of the kinetics on DHA. How does a *Gluconobacter* culture behave after a certain time of exposure to DHA?

Sattler (1964) already mentioned that the organism is not able to adapt to DHA, although it can do so to glycerol.

Gluconobacter oxydans was unable to grow in fresh medium after producing DHA for 25 h. This is found with sorbose fermentations. Thus, the cells must have lost viability due to dihydroxyacetone (or glycerol).

As shown in Fig. 2, the viability of *Gluconobacter* decreases exponentially with time of exposure to DHA. The rate of decrease depends on the DHA concentration in a linear manner. For the overall kinetics, this leads to a structured model with a biomass composed of viable cells (X_v) and non-viable cells (X_{nv}).This toxic effect of DHA can be modelled by a death rate kinetic:

$$dX_{nv}/dt = X_v \cdot \mu_{nv} \cdot P \quad (6)$$

$$X = X_v + X_{nv} \quad (7)$$

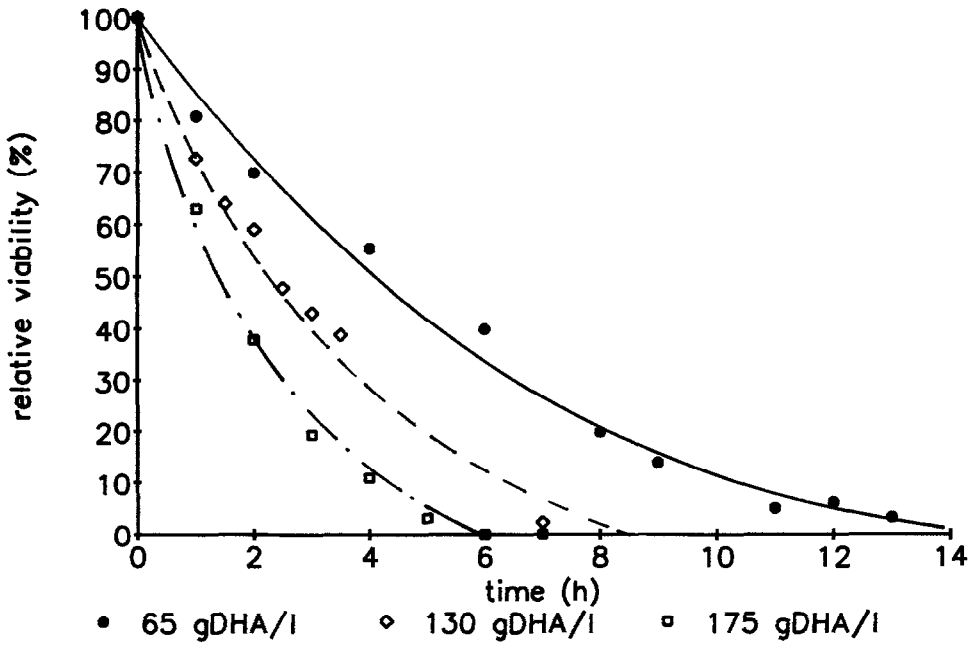

Fig. 2: Relative viability of *Gluconobacter* cells in DHA free medium after a certain time of exposure to DHA (the lines show graphical interpolations of measured values)

Additionally, a fermentation model has to account for cell lysis, which can be observed for extended fermentations. A correlation with dihydroxyacetone could not be found. Cell lysis is most likely to occur with non-viable cells. Thus, equation (6) has to be extended to:

$$dX_{nv}/dt = X_v \cdot \mu_{nv} \cdot P - \mu_l \cdot X_{nv} \qquad (8)$$

with μ_l as a lysis rate constant.

It still has to be determined how cell lysis depends on the availability of an energy source. It has been observed that a *Gluconobacter* culture tends to foam after glycerol or sorbitol as energy source is depleted. Foaming decreases again after feeding additional substrate.

The described results provide a fermentation model of the glycerol oxidation by *Gluconobacter oxydans*. The newly discovered death rate kinetics show the necessity of such a structured model. Only with the introduction of non-viable cells was it possible to model the dynamic behaviour of the system correctly.

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