High Enthalpy and Low Enthalpy Death in Saccharomyces cerevisiae *Induced by Acetic Acid*

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Acetic acid at concentrations as may occur during vinification and other alcoholic yeast fermentations induced death of glucose-grown cell populations of *Saccharomyces cerevisiae* IGC 4072 at temperatures at which thermal death was not detectable. The Arrhenius plots of specific death rates with various concentrations of acetic acid (0–2%, w/v) pH 3.3 were linear and could be decomposed into two distinct families of parallel straight lines, indicating that acetic acid induced two types of death: 1) High enthalpy death (HED) predominated at lower acetic acid concentrations (<0.5%, w/v) and higher temperatures; its enthalpy of activation ($\Delta H^{\neq}$) approached that of thermal death (12.4×10^4 cal/mol); 2) Low enthalpy death (LED) predominated at higher acetic acid concentrations and lower temperatures with $\Delta H^{\neq}$ of 3.9×10^4 cal/mol. While the $\Delta H^{\neq}$ values for HED induced by acetic acid were similar with those reported earlier for HED induced by other fermentation endproducts, the values for the entropy coefficients were different: 127–168 entropy units $mol^{-1}L$ for acetic acid as compared with 3.6–5.1 entropy units $mol^{-1}L$ for ethanol, which agreed with experimental results indicating that acetic acid is over 30-times more toxic than ethanol with respect to yeast cell viability at high process temperatures.

INTRODUCTION

Fermentation end-products other than ethanol may enhance the apparent toxicity of the latter for *Sacch. cerevisiae* with respect to growth, fermentation and viability.[1–3] Acetic acid is a normal end-product of fermentation by this yeast[3,4] and additional amounts may be produced by contaminating acetic acid bacteria.[5] Acetic acid is not metabolized by glucose-repressed yeast and enters the cell in the undissociated form by simple diffusion where it dissociates and, if the external pH is lower than the intracellular one, will accumulate as a function of ΔpH.[6,7]

During fermentation of grape must or other solutions of hexoses (including invert sugars which are rapidly produced when sucrose is the fermentation substrate) the yeast is repressed with respect to acetate metabolism.

Here evidence is presented that under such conditions acetic acid induces two types of death in *Sacch. cerevisiae* at concentrations as may occur during vinification and other alcoholic yeast fermentations.

MATERIALS AND METHODS

Strain

Saccharomyces cerevisiae IGC 4072 was originally isolated from a sample of Fermivin, an industrial wine yeast distributed by Rapidase, Selin, France. It was maintained on slants of glucose (2%, w/v), peptone (1%, w/v), yeast extract (0.5%, w/v), and agar (2%, w/v).

Viability experiments

Loss of viability of *Sacch. cerevisiae* populations induced by acetic acid was measured by methods described earlier for alkanol-induced death.[8]

RESULTS AND DISCUSSION

Experimental specific death rates (k_d) obtained at pH 3.3 in the presence and the absence of acetic acid were plotted as modified Arrhenius plots according to the theory of absolute reaction rates[9,10] (Fig. 1):

$$\ln \frac{k_d}{T} = \ln \frac{k_B}{h} + \frac{\Delta S^{\neq}}{R} - \frac{\Delta H^{\neq}}{R} \frac{1}{T} \qquad (1)$$

where T is absolute temperature, k_B is Boltzmann's constant, h is Planck's constant, R is the gas constant, $\Delta S^{\neq}$ is the entropy of activation and $\Delta H^{\neq}$ is the enthalpy of activation.

The combinations of temperature and concentrations of acetic acid were chosen in such a way that the experimental specific death rates were of the same order of magni-

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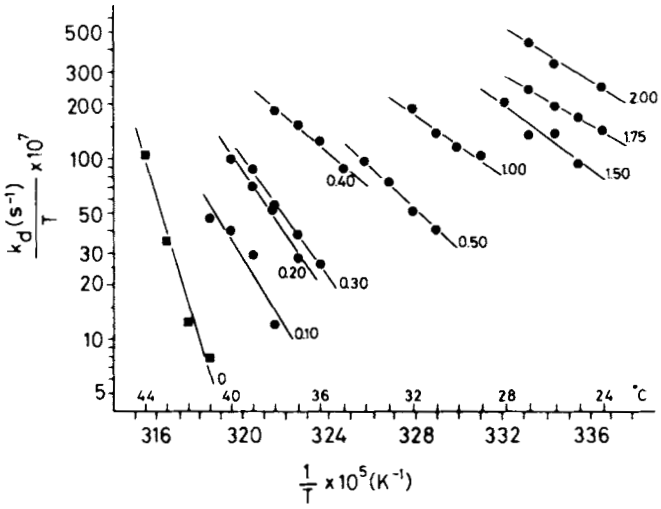

Figure 1. Dependence on the temperature of the specific death rates (k_d) of *Saccharomyces cerevisiae* IGC 4072 at pH 3.3 in the absence and the presence of acetic acid. Arrhenius plots according to eq. (1). Numbers indicate extracellular concentrations of acetic acid (%, w/v).

tude. Under these experimental conditions the absolute values of the slopes of the plots (i.e., $\Delta H^{\neq}/R$) decreased with increasing concentrations of acetic acid and decreasing experimental temperatures (Fig. 1). This suggested that two different types of death were induced by acetic acid: a high enthalpy death (HED) predominant at higher temperatures and lower acetic acid concentrations and a low enthalpy death (LED) predominant at lower temperatures and higher acetic acid concentrations. A similar behavior was observed earlier in *Sacch. cerevisiae* with respect to ethanol-induced death.[11]

Theoretical Arrhenius plots of LED (Fig. 2) were calculated using the following form of eq. (1):

$$\ln \frac{k_d}{T} = \ln \frac{k_B}{h} + \frac{\Delta S_0^{\neq} + C_E^A X}{R} - \frac{\Delta H^{\neq}}{R} \frac{1}{T} \qquad (2)$$

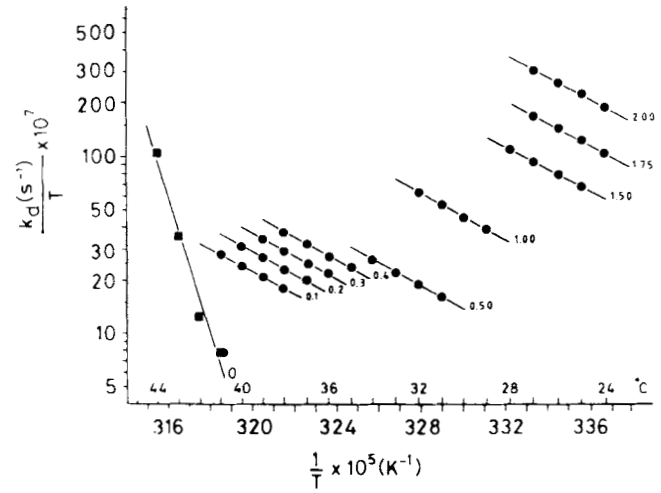

Figure 2. Theoretical Arrhenius plots calculated by the use of eqs. (2) and (3) of low enthalpy death of *Saccharomyces cerevisiae* IGC 4072 induced by acetic acid under the experimental conditions of Figure 1. Numbers indicate extracellular concentrations of acetic acid (%, w/v). (■) Experimental thermal death rates.

and

$$\Delta S_X^{\neq} = \Delta S_0^{\neq} + C_E^A X \qquad (3)$$

where $\Delta S_X^{\neq}$ is the entropy of activation of LED in the presence of concentration X of acetic acid in the medium and C_E^A the respective entropy coefficient (for a discussion of these entropy relations see Leão and van Uden[8]). Values for the different parameters used in the calculations were obtained as follows: $\Delta H^{\neq}$ was the average of the experimental $\Delta H^{\neq}$ values at pH 3.3 at concentrations 1.5, 1.75, and 2.0% (w/v) acetic acid. An estimate of entropy coefficient C_E^A was obtained from the linear isothermic death plot at 26°C, pH 3.3 (Fig. 4) according to the following equation:[8]

$$\ln k_d^X = \ln k_d^0 + \frac{C_E^A}{R} X \qquad (4)$$

where k_d^0 and k_d^X are the specific death rates in the absence and the presence of concentration X of acetic acid. Using this estimate and an experimental value of $\Delta S_X^{\neq}$ obtained at 2% (w/v) acetic acid, an estimate of $\Delta S_0^{\neq}$ was calculated by the use of eq. (3).

The theoretical Arrhenius plots of LED thus obtained (Fig. 2) were subtracted from the experimental plots. In this way a family of high enthalpy plots was obtained that represented HED death alone (Fig. 3). The plots were processed as described earlier for alkanol enhanced thermal death[8] and the following estimates where obtained: $\Delta H^{\neq} = 12.4 \times 10^4$ cal/mol a value similar with $\Delta H^{\neq}$ of thermal death (i.e., the value at 0% acetic acid) and C_E^A 168 entropy units mol^{-1} L. A similar estimate for C_E^A (127 entropy units mol^{-1} L) was obtained from an isothermal death plot at 39°C, pH 3.3 (Fig. 4).

It is concluded that HED death induced by acetic acid represented thermal death enhanced exponentially by the acid and that the toxic effect was reflected in the values of

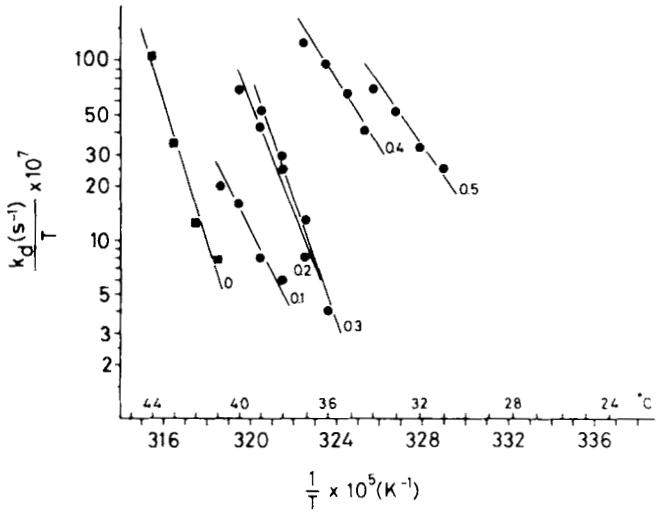

Figure 3. Theoretical Arrhenius plots, obtained by subtracting the plots of Figure 2 from the plots of Figure 1, of high enthalpy death in *Saccharomyces cerevisiae* IGC 4072 induced by acetic acid. Numbers indicate extracellular concentrations of acetic acid (%, w/v). (■) Experimental thermal death rates.

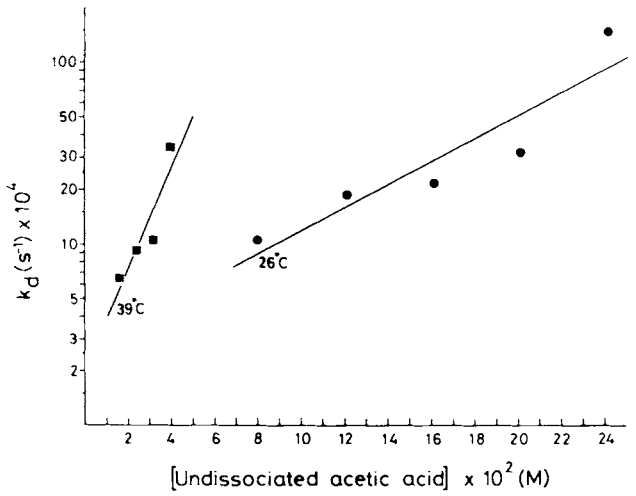

Figure 4. Dependence on the concentrations of extracellular acetic acid of the specific death rates (k_d) of *Saccharomyces cerevisiae* IGC 4072 at constant temperature and pH 3.3.

$\Delta S^{\neq}$ but not of $\Delta H^{\neq}$ (parallel Arrhenius plots). Furthermore acetic acid induced LED with similar characteristics but distinct from thermal death by its much lower $\Delta H^{\neq}$ (3.9×10^4 cal/mol) and C_E^A (24 entropy units mol^{-1} L).

HED is of practical importance since it contributes to so-called "heat-sticking" of alcoholic yeast fermentations particularly of red wine and fuel ethanol fermentations in warm countries in the absence of efficient temperature control.[12] As reported earlier ethanol and other alkanols[8] as well as octanoic and decanoic acids[2] also induced this type of cell death. While in all these cases the $\Delta H^{\neq}$ values were similar with $\Delta H^{\neq}$ of thermal death, the entropy coefficients had vastly different values (Table I). Introduction in eq. (4) of the entropy coefficients for HED induced by ethanol and by acetic acid led to the prediction that the latter is over 30-times more toxic, with respect to yeast cell viability at high process temperatures, than the latter (on a molar basis). This prediction is in agreement with experimental results (compare Figure 3 of the present article with Figure 1 of Leão and van Uden[8]). Thus these two fermentation endproducts may both contribute to significant HED at realistic concentrations and high process temperatures. A similar reasoning applies to the much more toxic octanoic and decanoic acids.[2]

With respect to LED acetic acid is also more toxic than ethanol (compare Figure 1 of the present article with Figure 2 of Sá-Correia and van Uden[11]). However, significant LED at intermediate and low temperatures requires rather high concentrations of the toxic metabolites which at least in the case of acetic acid are less realistic than the concentrations of acetic acid that induce significant HED at high process temperatures.

It remains to be seen which form(s) of acetic acid (acetate ion, undissociated acid or both) is(are) involved in HED and LED.

Table I. Entropy coefficients of the enhancement of high enthalpy death (thermal death) in *Saccharomyces* by alkanols and fatty acids.

Alkanols Fatty acids	Entropy coefficient (entropy units mol^{-1}L)	Species	Reference
Ethanol	3.6–5.1	*Sacch. cerevisiae*	8
Isopropanol	9.6–10.2	*Sacch. cerevisiae*	8
Propanol	15.3–17.4	*Sacch. cerevisiae*	8
Butanol	58.3–60.0	*Sacch. cerevisiae*	8
Octanoic acid	10.3×10^3	*Sacch. bayanus*	2
Decanoic acid	31.6×10^3	*Sacch. bayanus*	2
Acetic acid	127–168	*Sacch. cerevisiae*	present article

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