Improvement of Alcohol Fermentation of a Corn Starch Hydrolysate by Viscosity-Raising Additives

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Aim of this work is to investigate the simultaneous effects of viscosity and temperature on the productivity of the alcohol fermentation of starch hydrolysate by *Saccharomyces cerevisiae*. Batch fermentations have been carried out at given pH, broth composition, inoculum, and agitation intensity, but at varying temperature ($24 < T < 46\,°C$) or concentration of carboxymethyl cellulose ($0.4 < C_{CMC} < 2.0\,g/L$), chosen as viscosity-raising additive. The results of tests carried out at given viscosity demonstrate that the volumetric productivity increases with temperature up to an optimal value ($32–36\,°C$). At higher temperatures a productivity drop occurs. In addition, a viscosity increase up to about $12\,g\,m^{-1}\,s^{-1}$ (value determined at $30\,°C$) improves the fermentation kinetics, while the process is strongly negatively affected at viscosity values higher than this threshold. Both the Arrhenius and the so-called "thermodynamic" models have then been used to estimate the related thermodynamic quantities referred to both fermentation and thermal deactivation. A comparison of the values of these quantities suggests that both cell growth repression provoked by mass transfer limitations due to viscosity rise and the reduction of product inhibition are possible simultaneous causes of the observed productivity enhancement at low CMC levels.

1 Introduction

A significant influence of viscosity on the productivity of several bioprocesses is known since a long time. Generally, the productivity decreases with increasing viscosity of the fermentation medium. However, it has recently been demonstrated for alcohol fermentation of starch hydrolysate by *Saccharomyces cerevisiae* that, contrarily to processes forming highly viscous products, the addition of viscosity-raising additives to a common culture medium up to a certain threshold may result in relevant productivity rise [1–3].

Only a physical justification has been proposed up to now to explain this unexpected observation: the decrease in substrate (glucose) diffusivity due to the polymer layer around the cell could be greater than the product (ethanol) diffusivity decrease thus bringing about a reduction of product inhibition [4]. Unfortunately, however, an experimental verification of this hypothesis is virtually impossible, because of the difficulty in exactly reproducing the microenvironment around the free cells during metabolite diffusion tests. In contrast, relatively accurate diffusivity determinations have been made for different metabolites in the presence of biofilms, either natural [5, 6] or artificially prepared [7, 8].

Nevertheless, it is possible to indirectly shed light on the actual causes of this productivity rise by studying the eventual consequences, if any, of these mass transfer limitations on the kinetic parameters and thermodynamic quantities of the fermentation as well as on the physiological state of microbial cells. To this end, a complete set of batch fermentations of starch hydrolysate has been carried out at different temperatures and viscosities, using carboxymethyl cellulose (CMC) as viscosity-raising additive. The collected experimental data have then been elaborated to estimate the related kinetic parameters (maximum volumetric and specific productivities), which have been used as a comparison basis.

A previous study [4] clearly demonstrated that these kinetic parameters can be used, likewise enzymatic systems, to evaluate the thermodynamic quantities of alcohol fermentation, provided that a sufficient number of data at different temperatures is available. In this way, using standard procedures based on the Arrhenius model [9] or the so-called "kinetic" and "thermodynamic" approaches [10], the thermodynamic quantities of different enzymatic [9–12] or microbial systems [13–18] were estimated, taking into consideration not only the formation of the fermentation transition state but also the thermal deactivation.

The Arrhenius and the thermodynamic approaches are used in this study to estimate the same thermodynamic quantities in the presence of different levels of CMC (i.e. under different viscosity conditions), so as to ascertain the eventual thermodynamic nature of the observed effects on fermentation kinetics.

2 Materials and Methods

2.1 Microorganism

The cultures of *Saccharomyces cerevisiae* (GR-203) used in this study were maintained on agar-malt slants. The cells were grown aerobically at $30\,°C$ in shaken flasks on rotary shaker and harvested at the end of the exponential phase. Afterwards, the cells were aseptically inoculated into the fermentor $12\,h$ after they had been harvested.

2.2 Medium

The corn starch hydrolysate used in this study for broth preparation was kindly supplied by Roquette Italia SpA of Cassano Spinola, Italy. It was prepared by enzymatic hydrolysis of corn starch, as described in detail in previous papers [19, 20]. The average composition of the starch hydrolysate was: 59.1 % glucose, 1.8 % maltose, 0.49 % trisaccharides, 4.76 % polysaccharides, 2.6 % ashes, 0.73 % proteins, 30.5 % water, $0.305\,mg/g$ Ca^{2+}, $0.129\,mg/g$ Mg^{2+}, $71.50\,mg/g$ Na^+, and $1.09\,mg/g$ K^+. The medium used for cell growth and fermentation was obtained by diluting the starch hydrolysate with tap water up to the desired fermentable sugar concentration (about $38\,g/L$) and by adding the following salts: $5.0\,g/L$ KH_2PO_4, $2.0\,g/L$ $(NH_4)_2SO_4$, $0.4\,g/L$ $MgSO_4 \cdot 7\,H_2O$.

Fermentations at variable viscosity were performed by adding, according to the selected value of viscosity, the following amounts of CMC to $1.0\,L$ of broth: $0.4\,g$, $0.6\,g$, $1.0\,g$, $1.5\,g$, and $2.0\,g$.

2.3 Fermentation conditions

A 7.0-L Setric CSTR, with $5\,L$ working volume, stirred at $300\,rpm$, was employed for batch runs. The pH value of the fermentation broth was automatically regulated, to an accu-

racy of 0.1 pH units, by a pH control module Setric G.I., Set 7, provided with a peristaltic pump that injected a fine stream of 1 N NaOH solution. The temperature, automatically controlled to an accuracy of ± 0.1 °C by the same module, was varied between 24 and 46 °C. The fermentor and the medium were sterilised by autoclaving at 120 °C for 20 min.

All fermentations were carried out using an inoculum of 1.1 g dry weight/L. A few millilitres of the thick yeast suspension obtained by centrifugation, whose biomass concentration was previously determined as described below, were added to the medium up to the desired starting biomass concentration.

2.4 Analytical procedures

Ethanol production was followed by gaschromatography (Fractovap, model C, type ATC/t, Carlo Erba, Milan) with a column packed with Cromosorb W coated with Carbowax 1500. The column was kept at 130 °C and the detector at 190 °C. Helium was used as a carrier gas at a pressure of 1.5 bar. The gas chromatograph was calibrated several times during each run by means of standard ethanol-water solutions.

Cell concentration was determined by filtering a known volume of culture broth on 0.45 μm autoclavable filters. The filters were dried at 105 °C until no weight change between consecutive measurements was observed.

Glucose concentration was determined by the Böhringer enzymatic method Cat. No. 139041 [21]. Maltose concentration was expressed as glucose and was calculated by the same method after hydrolysis of maltose to glucose by using α-glucosidase.

The kinematic viscosity of the different solutions was determined by calibrated Ostwald viscosimeters. For this purpose – following the suggestion of *Gholap* et al. for hydroxymethyl cellulose solutions up to 10 g/L [22] – a Newtonian behaviour has been supposed for all solutions within the whole experimental range of CMC concentration tested in this study. The density was determined in glass cylinders by liquid level displacement provoked by the immersion of calibrated densimeters into the solutions. The dynamic viscosity has then been estimated as the product of the kinematic viscosity and the relative density. Both properties were determined at different temperatures by immersing the viscosimeters or the cylinders of densimeters into the water bath of a Tamson TCV45 thermostat connected to a Tamson TK20 refrigerator.

3 Theoretical Considerations

The thermodynamic quantities of alcohol fermentation have been estimated using either a graphic procedure based on the Arrhenius model [9], which allows to separately obtain activation enthalpy and entropy for fermentation and thermal deactivation, or the so-called thermodynamic approach [10], which resorts only to one equation including all thermodynamic quantities.

3.1 Arrhenius model

The model is based on the Arrhenius equation describing the general dependence of reaction rate constant (k) on temperature (T):

$$k = A \exp(-\Delta H^*/RT) \tag{1}$$

where ΔH^* is the activation enthalpy, R the ideal gas constant, and A the Arrhenius pre-exponential factor linked to the activation entropy.

In bioprocesses using microorganisms, Eq. (1) can be written in the form:

$$(dP/dt)_{max} = AXY_{P/X} \exp(-\Delta H^*/RT) \tag{2}$$

where $(dP/dt)_{max}$ is the maximum value of volumetric productivity, X the cell mass concentration, and $Y_{P/X}$ the yield of product per biomass.

The values of $(dP/dt)_{max}$ usually describe curves including a first tract where the rate constant increases with temperature up to an optimal value (T_{opt}), which is consistent with the activated complex theory, and a second one where the rate constant decreases due to thermal deactivation. In enzymatic systems this decrease is certainly due to denaturation of the enzyme, whereas in whole cell systems it could be ascribed either to cell disfunction or to deactivation of the enzyme controlling a fundamental metabolic pathway.

This behaviour can then be described by two Arrhenius-type straight lines:

$$\ln(dP/dt)_{max} = \ln (AXY_{P/X}) - \Delta H^*/RT \quad \text{For } T < T_{opt} \tag{3}$$

$$\ln(dP/dt)_{max} = \ln (BXY_{P/X}) - \Delta H^{*'}/RT \quad \text{For } T > T_{opt} \tag{4}$$

and

$$\Delta H^*_D = \Delta H^* + |\Delta H^{*'}| \tag{5}$$

where ΔH^*_D and B are the activation enthalpy and a sort of pre-exponential factor for thermal deactivation, respectively.

The activation enthalpies (ΔH^* and ΔH^*_D) and the Arrhenius pre-exponential factors (A and B) of both fermentation and thermal deactivation have been estimated in this study from the slopes and the intercepts on the ordinate axis of these straight lines, while the related activation entropies (ΔS^* and ΔS^*_D) have consequently been estimated with the equations:

$$\Delta S^* = R \ln (Ah/k_B T) \tag{6}$$

$$\Delta S^*_D = R \ln (Bh/k_B T) \tag{7}$$

where k_B and h are Boltzmann's and Planck's constants respectively.

3.2 Thermodynamic approach

If the thermal deactivation of microbial metabolism is the result of the denaturation of an enzyme controlling a fundamental metabolic pathway, an instantaneous equilibrium between the native (E_N) and denatured (E_D) forms of this enzyme can be assumed:

$$E_N \underset{\rightleftharpoons}{K_D} E_D \tag{8}$$

where the equilibrium constant K_D is a function of temperature according to:

$$K_D = \exp (\Delta S^\circ_D/R) \exp(-\Delta H^\circ_D/RT) \tag{9}$$

ΔS°_D and ΔH°_D being the standard variations of entropy and enthalpy, respectively.

The overall dependence of the maximum productivity of a bioprocess on temperature is then given by:

$$(dP/dt)_{max} = \frac{AXY_{P/X} \exp(-\Delta H^*/RT)}{[1 + C \exp(-\Delta H^\circ_D/RT)]} \tag{10}$$

where C is the entropy contribution of the deactivation equilibrium.

With the help of a personal computer using the Table Curve Jandel Scientific (a fitting programme by least square error), the experimental data of $(dP/dt)_{max}$ have been used to estimate the values of the four thermodynamic quantities appearing in this equation ($A, C, \Delta H^*$ and ΔH°_D).

4 Results and Discussion

4.1 Kinetic results

Batch fermentations of corn starch hydrolysate have been carried out at different temperatures ($24 < T < 46\,°C$) and carboxymethyl cellulose concentrations ($0 < C_{CMC} < 2.0\,g/L$), using a constant starting biomass level of 1.1 g (d.w.)/L.

Experimental data of biomass, product and substrate concentrations collected during these runs have been used to calculate the values of maximum volumetric productivity listed in Tab. 1. These results show that maximum productivity regularly increases with temperature at a given level of this viscosity-raising additive up to a maximum temperature threshold. Then, a sharp fall takes place likely due to thermal denaturation of the enzyme supposed to control one of the fundamental metabolic pathways. The temperature at which productivity reaches this threshold also seems to depend on CMC level, showing a maximum of 36 °C at $C_{CMC} = 0.4\,g/L$ and a minimum of 32 °C at $C_{CMC} = 0.6–1.0\,g/L$.

These remarkable effects of CMC level on fermentation kinetics can reasonably be associated with the related viscosity increase, on the basis of the following reasoning. As Figs. 1 and 2 clearly show, the progressive addition either of glucose or CMC in the culture medium is characterised by nearly linear increases in dynamic viscosity. These results well coincide with those recently presented for aqueous solutions of glucose and CMC [23]. A model derived from the well-known Guzman-Andrade equation [24] has successfully been used in that work to describe the linear contribution of each solute to viscosity as well as the exponential decrease of this parameter with temperature. According to these results, it seems reasonable to ascribe in the following sections any variation of the thermodynamic quantities, consequent on CMC addition, to the related viscosity rise.
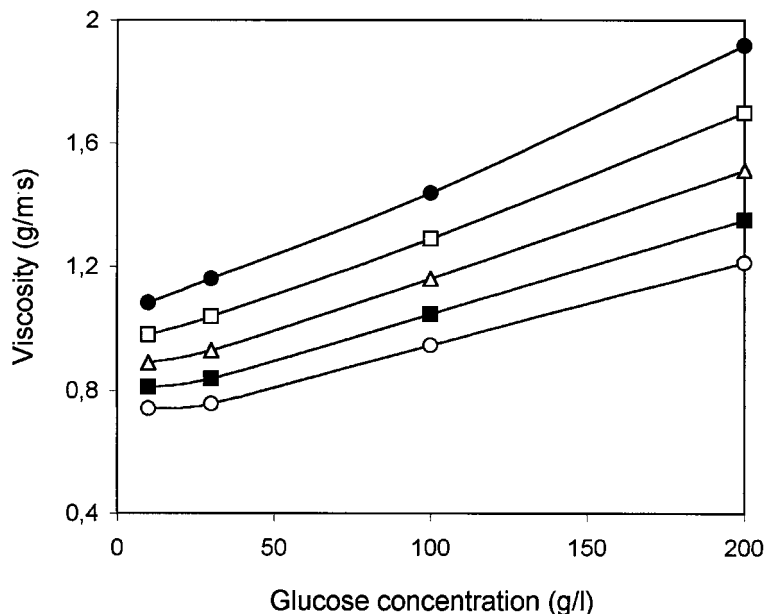
Tab. 1. Maximum productivity values calculated for batch fermentations of corn starch hydrolysate by *S. cerevisiae*, carried out at different temperatures and carboxymethyl cellulose concentrations.

C_{CMC} (g/L)	0.4	0.6	1.0	1.5	2.0
T (°C)					
24	1.30	1.80	2.00	1.55	1.51
25	1.41			1.70	1.60
26			2.20	1.82	
27		2.09	2.30		1.90
28	1.64	2.25		2.09	
29		2.30	2.45		2.15
30	1.94	2.45	2.55	2.25	2.30
31		2.55	2.70		2.45
32	2.08	2.67	2.85	2.55	
33					2.70
34	2.29	2.50	2.65	2.70	2.65
35					2.48
36	2.40	2.40	2.50	2.55	
37					2.15
38	2.05	2.30	2.25	2.25	
39			2.20		1.90
40	1.90	2.20	2.10	2.00	
41	1.78		2.00		1.65
42		2.00	1.90	1.70	
43	1.65			1.65	1.50
44	1.60			1.57	
45					1.30
46	1.44	1.87			

Starting from the experimental values of $(dP/dt)_{max}$, enthalpies and entropies of alcohol fermentation and thermal deactivation have been estimated using both the Arrhenius model (Fig. 3) and the equilibrium approach (Fig. 4). The values of these thermodynamic parameters estimated with these models are listed in Tabs. 2 and 3, respectively, and discussed in the following sections.

4.2 Thermodynamic parameters estimation

The typical productivity behaviours shown in Fig. 3 are similar to those commonly observed for maximum specific growth rate of different organisms [13], although the activation energies (ranging from about 32 to 51 kJ/mol) presented in this study (Tab. 2) are quite lower than those observed for microbial growth (54–71 kJ/mol). While the fall observed

Fig. 1. Effect of glucose concentration on the dynamic viscosity of starch hydrolysate solutions. T (°C): (●) 20; (□) 25; (△) 30; (■) 35; (○) 40.

Tab. 2. Thermodynamic parameters estimated by the Arrhenius model for batch fermentations of corn starch hydrolysate carried out at different carboxymethyl cellulose concentrations.

C_{CMC} (g/L)	0.4	0.6	1.0	1.5	2.0
Fermentation					
ΔH^* (kJ/mol)	40.3	37.2	31.9	41.9	50.6
ΔS^* (kJ mol^{-1} K^{-1})	−0.20	−0.21	−0.22	−0.19	−0.16
[a]r^2	0.993	0.994	0.985	0.995	0.999
Thermal deactivation					
ΔH^*_D (kJ/mol)	80.1	58.0	64.1	88.8	102.8
ΔS^*_D (kJ mol^{-1} K^{-1})	−0.21	−0.28	−0.24	−0.19	−0.17
[b]r^2	0.996	0.985	0.997	0.991	0.986

[a] Determination coefficients referred to the straight lines described by Eq. (3).

[b] Determination coefficients referred to the straight lines described by Eq. (4).

for the maximum growth rate above the optimal temperature for growth was ascribed to an increased rate of microbial death [13], the productivity drop observed in this work could only be explained by an increased denaturation rate of one controlling enzyme.

Tab. 3. Thermodynamic parameters estimated by the equilibrium approach for batch fermentations of corn starch hydrolysate, carried out at different carboxymethyl cellulose concentrations.

C_{CMC} (g/L)	0.4	0.6	1.0	1.5	2.0
Fermentation					
ΔH^* (kJ/mol)	76.0	76.1	75.3	75.7	75.3
ΔS^* (kJ mol^{-1} K^{-1})	−0.078	−0.077	−0.077	−0.077	−0.075
Thermal deactivation					
ΔH^*_D (kJ/mol)	194.4	193.7	192.9	193.5	193.1
ΔS^*_D (kJ mol^{-1} K^{-1})	0.626	0.626	0.626	0.626	0.626
K_D	0.044	0.059	0.082	0.063	0.074
[a]r^2	0.853	0.079	0.774	0.960	0.945

[a] Determination coefficients referred to the curves described by Eq. (10).

Likewise the death rate, also the enzyme denaturation rate, is a strong function of temperature, being characterised by high activation enthalpies (60–100 kJ/mol, see Tab. 2). These high values mean that the rate of enzyme denaturation increases much faster with temperature than the product formation rate and that the overall productivity declines above its maximum value. Nevertheless, these activation enthalpies
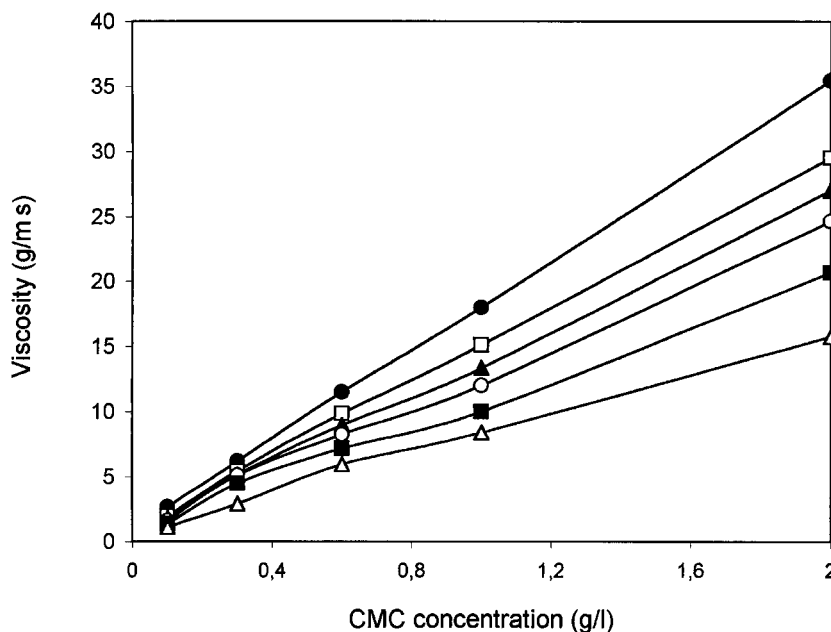

Fig. 2. Effect of CMC concentration on the dynamic viscosity of starch hydrolysate solutions. T (°C): (●) 16; (□) 22; (▲) 26; (○) 30; (■) 38; (△) 48.
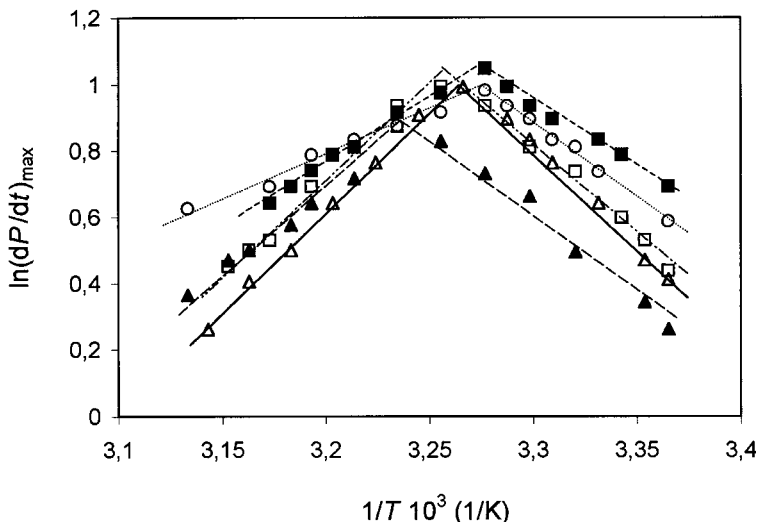

Fig. 3. Graphical estimation of the thermodynamic parameters of both fermentation and thermal deactivation according to the Arrhenius model. C_{CMC} (g/L): (▲) 0.4; (○) 0.6; (■) 1.0; (□) 1.5; (△) 2.0.
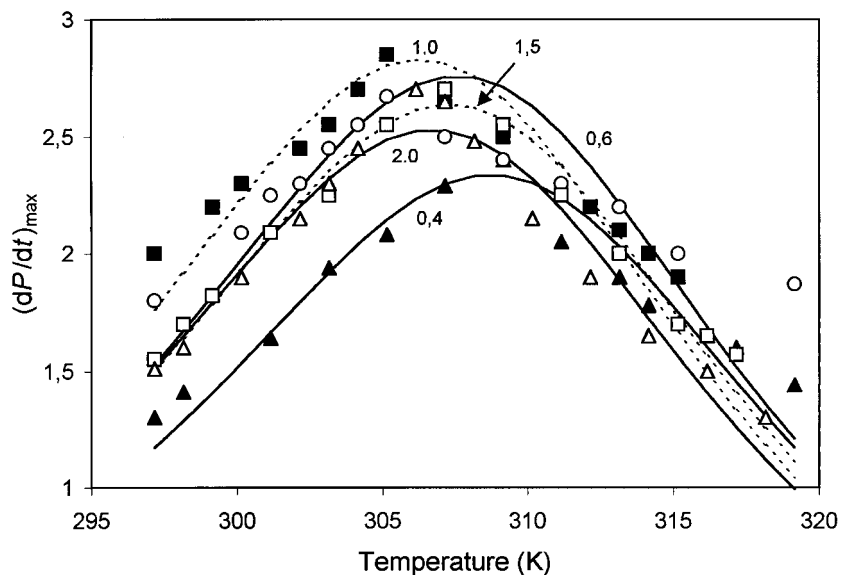
Fig. 4. Estimation of the thermodynamic parameters of both fermentation and thermal deactivation according to the thermodynamic approach. C_{CMC} (g/L): (▲) 0.4; (○) 0.6; (■) 1.0, (□) 1.5; (△) 2.0.

are obviously lower than those reported for microbial death (290–380 kJ/mol) [13].

As stated in a previous work [4], unfortunately not all the values calculated with the Arrhenius and the thermodynamic approaches are directly comparable, because they often refer to dishomogeneous quantities: in fact, while ΔH^*_D and ΔS^*_D refer to an activation state consistent with the Arrhenius theory of the activated complex, ΔH°_D and ΔS°_D are standard variations of a reversible deactivation equilibrium. In addition, the estimation of the activation entropies of both fermentation and thermal deactivation, using the Arrhenius approach, refer to a reference temperature of 25 °C, these thermodynamic parameters being slightly dependent on temperature when estimated from A and B. So, a comparison must be restricted only to homogeneous quantities.

4.2.1 Arrhenius model

Contrary to what evidenced in a previous work [4], where the thermodynamic parameters were calculated in the absence of carboxymethyl cellulose and without considering the product yield per biomass ($Y_{P/X}$), the values of activation enthalpies here estimated for fermentation through the Arrhenius approach are comparable with those found in the literature for a variety of enzymatic systems [9–12, 25]. This confirms not only the necessity of using $Y_{P/X}$ in both Eqs. (3) and (4) for the estimation of fermentation thermodynamic parameters but also the consistency of considering thermal deactivation as a phenomenon related to the denaturation of a controlling enzyme. A further confirmation of this assumption comes from the values of the activation entropy estimated when the product yield per biomass is taken into account. These values have the same negative sign not only as those estimated by the thermodynamic approach but also as those calculated by *Sizer* [25] for some specific reactions catalysed by enzymes, which is consistent with the formation of an enzyme-substrate complex implying a reduction of the system randomness during the complex formation. No discrepancy with the activated complex theory is then evidenced when $Y_{P/X}$ is used in this approach.

4.2.2 Thermodynamic approach

As far as the estimation of fermentation thermodynamic parameters by the so-called thermodynamic approach is concerned, its reliability was demonstrated in the absence of car-

boxymethyl cellulose as viscosity-raising agent by simulating parametric variations of every thermodynamic quantities [4].

With particular reference to the fermentation thermodynamic parameters, it was put in evidence under those conditions that a productivity increase can be the result of a) an increase in biocatalyst concentration (X), b) an increase in the probability of effective collisions between substrate and biocatalyst (A), c) a decrease in the activation enthalpy (ΔH^*), and d) an increase in the activation entropy (ΔS^*) which would imply, according to the Gibbs-Helmholtz equation, a consequent reduction of the free enthalpy of activation. On the light of the introduction of the yield of product per biomass in Eqs. (3) and (4) to improve their reliability in thermodynamic quantity estimation, this parameter could be recognised as an additional factor positively influencing fermentation productivity. In fact, an increase in $Y_{P/X}$ implies a reduction of the substrate fraction utilised for the growth, which becomes obviously available for product formation.

On the other hand, making reference to the thermodynamic parameters of the deactivation equilibrium only, it was shown that a productivity fall can be the result of an increase in standard entropy (ΔS°_D) – which would lead to a corresponding decrease in standard free enthalpy – or of a decrease in the biocatalyst active fraction. This effect was obviously evidenced only at temperatures higher than 30 °C at which thermal deactivation becomes significant. Any increase in ΔH°_D, on the other hand, was recognised as a factor opposing to the deactivation equilibrium, thus enhancing the fermentation kinetics.

4.3 Effect of CMC on thermodynamic parameters

Having now a look at the values of the thermodynamic parameters estimated at five different viscosities, corresponding to five different levels of CMC (Tabs. 2 and 3), it should be noted that the thermodynamic approach gives always higher fermentation activation enthalpies and always lower activation entropies (in absolute values) than the Arrhenius approach. Although a comparison between the thermodynamic parameters for thermal deactivation estimated by these different approaches would not be rigorous, because it would refer to different hypotheses (activated complex formation and deactivation equilibrium), the thermodynamic approach seems to give always positive entropy values and
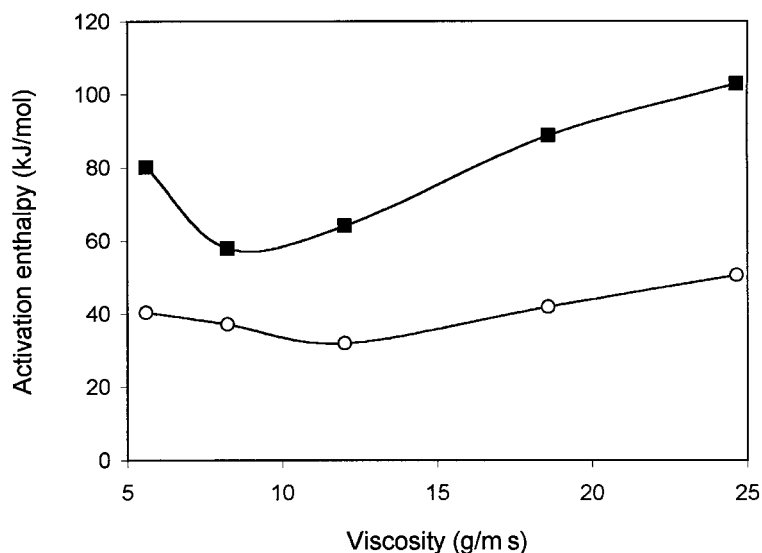
Fig. 5. Viscosity dependence of the activation enthalpies of (○) fermentation and (■) thermal deactivation, estimated with the Arrhenius model.

remarkably higher enthalpy values than the Arrhenius approach.

The most significant result coming from this comparison is, however, the nearly absolute constancy of all the thermodynamic quantities when using the thermodynamic approach, while the Arrhenius model appears to be much more sensitive to productivity variations associated with the carboxymethyl cellulose level. Nevertheless, the values listed in Tab. 1 seem to evidence that maximum productivity is significantly influenced by both viscosity and C_{CMC}, which means that the Arrhenius model is more suitable for the estimation of fermentation thermodynamic parameters (provided that $Y_{P/X}$ is used), while the thermodynamic approach is better for enzymatic systems which are more sensitive to thermal deactivation. This consideration is also supported by the considerably higher values of the square of the correlation coefficient (r^2) obtained in this work with the Arrhenius model.

Thus, plotting in Fig. 5 the activation enthalpies of fermentation and thermal deactivation estimated only by the Arrhenius model versus viscosity, it can be seen that both quantities initially decrease up to a minimum value (corresponding to $C_{CMC} = 1.0\,g/L$ and $0.6\,g/L$, respectively) and afterwards rise with increasing viscosity. This means that low levels of CMC favour the fermentation by reducing its activation enthalpy but, at the same time, favour also the thermal deactivation equilibrium. Beyond the thresholds of $1.0\,g/L$ and $0.6\,g/L$ high levels of CMC show an opposite effect.

By analysing these effects on the whole, it seems reasonable to conclude that the stimulating effect of CMC levels not exceeding 1 g/L on fermentation could be the result of the optimum compromise between two different phenomena which contrarily act on productivity with increasing viscosity. It is evident, from the best performances observed in Tab. 1 at $C_{CMC} = 1\,g/L$, that the effect on ΔH^* exerted by CMC (or viscosity) is more important than the one detected for ΔH^*_D. In other words, CMC seems to improve the fermentation kinetics through a decrease in the activation enthalpy of fermentation rather than through a protection of the biocatalyst against thermal deactivation. This seems to be confirmed by the values of the equilibrium constant of the deactivation equilibrium (Eq. 9) reported in Tab. 3, which reveal that a biocatalyst fraction ranging only from 4 to 8 % can be considered thermally inactivated according to this approach.

A possible explanation of the progressive decrease of ΔH^* with viscosity (up to a minimum value) was provided in a previous study: the decrease in substrate (glucose) diffusivity, due to the polymer layer around the cell generated by CMC, could be greater than that of product (ethanol), thus bringing about a reduction of product inhibition [4]. At higher CMC levels, this situation could be reversed. It is difficult, on the other hand, to provide, on the basis of the actual knowledge, a justification for the decreasing protection against thermal deactivation detected in the same C_{CMC} range.

List of symbols

A	Arrhenius pre-exponential factor of fermentation, h^{-1}
B	Arrhenius pre-exponential factor of thermal deactivation, h^{-1}
C	entropy contribution appearing in Eq. (10)
C_{CMC}	carboxymethyl cellulose concentration, g_{CMC}/L
h	Planck's constant, kJ · h
ΔH^*	activation enthalpy of fermentation, kJ/mol
$\Delta H^{*'}$	thermodynamic quantity defined in Eq. (4), kJ/mol
ΔH^*_D	activation enthalpy of thermal deactivation, kJ/mol
k	reaction rate constant, h^{-1}
k_B	Boltzmann's constant, kJ/K
K_D	equilibrium constant of the reversible deactivation reaction
μ	dynamic viscosity, g m^{-1} s^{-1}
P	product (ethanol) concentration, g_P/L
R	ideal gas constant, kJ mol^{-1} K^{-1}
ΔS^*	activation entropy of fermentation, kJ mol^{-1} K^{-1}
ΔS^*_D	activation entropy of thermal deactivation, kJ mol^{-1} K^{-1}
t	fermentation time, h
T	temperature, °C or K
X	biomass concentration, g/L
$Y_{P/X}$	yield of product per biomass, g_P/g_X
ΔS°_D	standard variation of thermal deactivation entropy, kJ mol^{-1} K^{-1}
ΔH°_D	standard variation of thermal deactivation enthalpy, kJ/mol

Subscripts

CMC	carboxymethyl cellulose
max	maximum value
opt	optimum value
P	product
X	biomass

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