Achieving Procedure of a Kinetic Model to Predict the Influence of the Process Global Temperature on a Technological Alcoholic Fermentation

II. An Arrhenius type Kinetic Model

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The aim of the present work is to offer a practical methodology to realise an Arrhenius type kinetic model for a biotechnological process of alcoholic fermentation based on the Saccharomyces cerevisiae yeast. Using the experimental data we can correlate the medium temperature of fermentation with the time needed for a fermentation process under imposed conditions of economic efficiency.

Keywords: alcoholic fermentation, Arrhenius type kinetic model

Ethanol is a product obtained by a fermentative process in presence of the *Saccharomyces cerevisiae* yeast, its biosynthesis being based on transforming the monosaccharide into alcohol [1] according to the reaction:

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH \qquad (1)$$

Temperature is an important parameter in this bioprocess due to its influence on the biomass growth rate, on the glucose substrate transformation into a final product and on the resulting biomass composition [2–4]. In the first part of this paper we presented the experimental data obtained in three alcoholic fermentations of the *Saccharomyces cerevisiae* yeast on a technical substrate of hydrolysed starch. These experiments were carried out at different temperatures, their monitoring being realised by a modest endowment [5, 6]. The purpose of the present paper is to demonstrate that there is a forecast method of the time necessary for an alcoholic fermentation process. It is important to note here that the experimental data of fermentations, their pursuance and recording were performed through a simple procedure applied to yeast, sugar and water. For the present study we used a classical methodology of Arrhenius type. It is extremely important in industry that the benefit should be maxim and, to it obtain there are used higher productivity processes. Considering that at the end of the bioprocess the biosynthesis rate of the primary metabolite was falling suddenly due to certain reasons we focused on the part of the fermentative process which pointed to an acceptable production of ethanol.

Experimental part

In the first part of this paper we presented in detail the monitoring experimental procedure of the working temperature, [6]. The influence of the temperature on alcoholic fermentation was proved analyzing the substrate S, as a carbon – energy source, the biosynthesized ethanol P, as fermentation principal product and the dried cellular biomass X.

The monitoring was based on the initial and final gravimetric balances of the bioprocesses, on recording the emitted CO_2 flows and on determining the sugar and biomass contents. The content of the alcohol obtained in these bioprocesses was further determined. The final procedure of the studied processes is different in these two parts of the paper. In the first part, the final moment of the fermentative process was picked out arbitrary, respectively the moment when the emitted CO_2 flow was diminished under 1 $mL \cdot min^{-1}$. In this present part, without to diminish appreciably the intensive indicators of the bioprocesses, we introduced the global gravimetric productivity, P_m^{fin}, for a process reduced towards the integral fermentation.

Results and discussions

With a view to realize this study we used a parameter with a higher objectivity, respectively the sequential gravimetric productivity, P_m^s which represents the quantity of alcohol biosynthesized in the time unit by a quantity of fermentative biomass. Its measure units are (ethanol mg) · (fermentative mass kg)$^{-1}$· (fermentation hour)$^{-1}$.The principal element of temporal supervision for the three fermentations was the monitoring of the emitted CO_2 flow. This procedure was presented in [6]. We selected an arbitrary value for the sequential gravimetric productivity because this parameter was correlated with the biosynthesized ethanol. This value was calculated for a time of a half-hour, respectively the interval between two successive determinations of the emitted CO_2 flow. In this interval the bioprocess could be considered exhausted, inefficient economically, and in P_m^{fin} this moment is noted by superscript "*fin*". In our experiments the value was of 50 $mg \cdot kg^{-1} \cdot h^{-1}$. So, the moment when the emitted CO_2 flow in bioreactor was diminished at a level which corresponds to the final sequential productivity should be considered as a post-factum final moment. From practical points of view we continued the experiments until it was obtained an arbitrary flow of about 1 mL $CO_2 \cdot min^{-1}$.

With the purpose to identify the final moment of the bioprocess which corresponds to a higher economic efficiency we realised an estimation computation using a graphical and analytical interpolation. For this, in the experimental data which represent the evolutions of the biotechnological parameters, (represented in the figures 1-3 through dash lines) we marked this moment for every fermentative process.

In table 1 there are included the values of the principal biotechnological parameters, values calculated also through interpolation, for a interval of 30 min. This time represents the interval between two successive measurements of the emitted CO_2 flow, for those temporal

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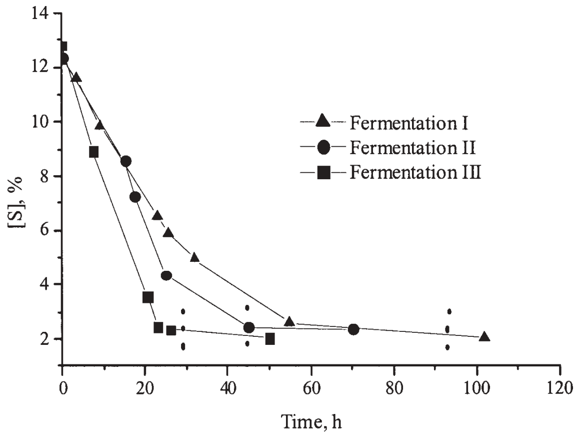

Fig. 1. The variation in time of the substrate concentration

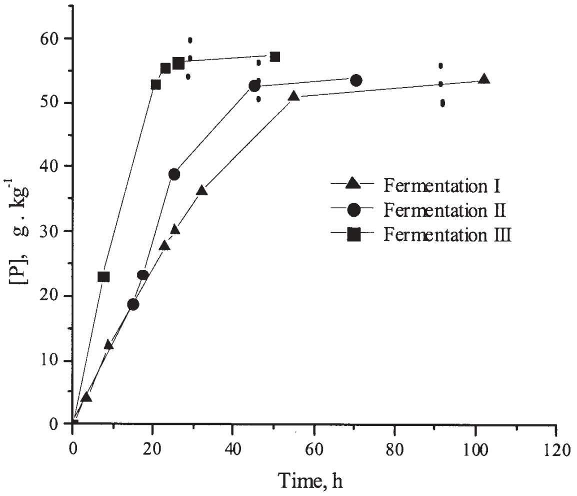

Fig. 2. The variation in time of the gravimetric concentration of the biosynthesized alcohol

points where the final sequential productivity had the value of 50 ethanol mg · (fermentative mass kg)$^{-1}$ · (fermentation hour)$^{-1}$. In the last four columns from table 1 are registered, in brackets, the percentage modifications of the biotechnological parameters, determined for the studied fermentative processes. Analysing these differences one can observe an important appearance. First, for the yield of global product, $Y_{P/S}$ and the two energetic yields experimental, (η^{exp}) and technological (η^{tech}), defined in [6], the variations are minimum – positive or negative – in general below 1.5%. For η^{tech} the values are always negative, while for $Y_{P/S}$ and η^{exp} the values are identical, positive for the fermentations III and I, and negative for the fermentation II.

In the case of the global gravimetric productivity, P_m^{fin}, for a reduced process based on an economic criterion toward the integral fermentation, there were registered important variations. These are of 9.13% for the fermentation I, 59.39% for the fermentation II and 76.03% for the last fermentation. Analyzing these differences we concluded that these values represent the material proof of the possibility of a substantial enlargement of the extensive technical-economic indicator, respectively the global gravimetric productivity of the bioprocess. It is important to mention that the working temperature was increased up to 35°C because this value represents the superior limit for the majority of the yeast mesophiles.

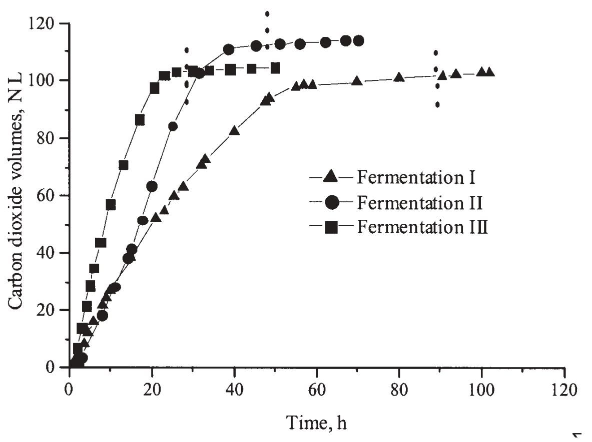

Fig. 3. The variation in time of obtained carbon dioxide volumes $\Sigma V^o_{CO_2}$

The determination of the thermo-kinetic parameters

If we are starting from the theoretical premise that the global reaction (1) is a reaction of order one, respectively there is a rate constant represented by the equation (2):

$$k=\frac{1}{t}\ln\frac{C_o}{C^{fin}} \tag{2}$$

This equation can be obtained through the integration of the differential equation (3):

$$-\frac{dC}{dt}=k\cdot C \tag{3}$$

In accordance with the data from table 1, we can correlate the rate constant with the working mean temperature based on Arrhenius type dependence. This dependence could predict the efficiency of other alternatives for such bioprocesses realised in the same conditions of concentrations using different working average temperatures.

The working procedure is classic implying the determination of the activation energy and of the pre-exponential factor from the Arrhenius equation:

$$k=k_o\cdot e^{-\frac{E_A}{R\cdot T}} \Rightarrow \ln k=\ln k_o-\frac{E_A}{R}\cdot\frac{1}{T} \tag{4}$$

Table 1

THE FINAL BIOTECHNOLOGICAL PARAMETERS OF THE REALISED FERMENTATIONS

Number fermentation	Final average time, h, min	Final average emperature of fermentation, °C	P_{et}, g	S_{fin}, g	ΔS^{fin}, g	$Y^{fin}_{P/S}$, $g\cdot g^{-1}$	P_m^{fin}, $g\cdot kg^{-1}\cdot h^{-1}$	η^{fin}_{exp}, %	η^{fin}_{tech}, %
I	92 h 49.75'	22.59	198.70	79.44	408.83	0.486 *(+0.39%)*	0.555 *(+9.13%)*	95.03 *(+0.39%)*	79.57 *(-0.63%)*
II	42 h 56.36'	29.89	196.78	91.09	403.68	0.488 *(-1.06%)*	1.184 *(+59.39%)*	95.31 *(-1.06%)*	77.77 *(-1.55%)*
III	28 h 0.19'	34.73	212.50	85.88	426.60	0.498 *(+1.05%)*	1.956 *(+76.03%)*	97.41 *(+1.05%)*	81.08 *(-1.25%)*

Table 2

THE THERMO-KINETIC DATA OF THE THREE FERMENTATIONS TEMPORAL REDUCED ACCORDING TO AN ECONOMIC CRITERIA

Number fermentation	Time of fermentation, s	Fermentative mass, g	$[S]^{fin}$, %	Average temperature of fermentation, K	1/T, k^{-1}	k, s^{-1}	ln k
I	$3.342 \cdot 10^5$	3715.51	2.14 *(+5.58%)*	295.74	3.381×10^{-3}	5.213×10^{-6}	-12.164
II	$1.546 \cdot 10^5$	3742.15	2.43 *(+3.40%)*	303.04	3.300×10^{-3}	1.051×10^{-5}	-11.463
III	$1.008 \cdot 10^5$	3756.56	2.29 *(+12.91%)*	307.88	3.248×10^{-3}	1.709×10^{-5}	-10.977

Using a temporal limitation of other alternative fermentations, processed until the moment when the processes were inefficient, we obtained the thermo-kinetic data which are included in table 2.

Applying the linear regression procedure (i.e. the smallest squares method [7]) we obtained for the activation energy the value: E_A = 73.846±1.679 kJ $\cdot mol^{-1}$, while the pre-exponential factor varied exponential between $2.931 \cdot 10^7$ s^{-1} and $1.116 \cdot 10^8$ s^{-1} with an exponential average value of $5.719 \cdot 10^7$ s^{-1}, corresponding to the magnitude ln k_o of 17.862±0.668.

Also, for the statistical parameters which characterize the linear degree of the model we obtained the values:

- the dispersion S_y = 1.919. 10^{-2};
- the standard deviations:
  - slope standard deviation s_a = 201.894 (*2.27%*)
  - standard deviation of the cut to origin s_b = 0.668 (3.74%).

The most representative expression was the absolute value variation of the linear correlation coefficient, **r,** versus the unity. Its size, of - 0.9997, i.e., 1- |r | = 2.583 . 10^{-4}, (258.3 ppm), is a satisfactory result for the linearity of the model. This linear correlation is concluded in figure 4.

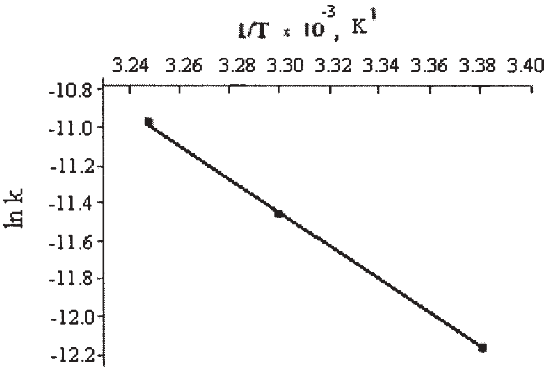

Fig. 4. ln k variation toward 1/T for the studied fermentations

Conclusions

In this part of the work we had in view the determination of the thermo-kinetic parameters using a model of Arrhenius type. Based on the experimental data, we correlated the fermentation average temperature with the time needed for the fermentation in imposed conditions of economical efficiency. First, it was taken as the final moment of the bioprocess the arrival at a sequential productivity for a half-hour between two measurements as being 50 mg$\cdot kg^{-1} \cdot h^{-1}$. This value represents 25 mg alcohol biosynthesized by 1 kg fermentative biomass during a half hour. The experimental data showed that, reducing the fermentations time with the last stages, considered as being economical inefficient, the technical – economic parameters of the bioprocesses were not affected considerably. For the integral fermentations we totalized now the next values and in brackets we present the reduction percentages of the initial time of the fermentations:

I : 9 h, 10.25 min (8.99 %)
II : 27 h, 3.64 min (38.66 %)
III : 21 h, 59.81 min (43.99 %)

So that, reducing the total time of the fermentative mass kept in bioreactor, we realized an efficiency of the fermentation process and for the yields $Y_{P/S}^{fin}$ and η_{exp}^{fin} we obtained values modified in limits of about ±1%, while for $\eta_{tech.}^{fin}$ the value was modified with maximum 1.55%, in the case of fermentation II. Also, the content of residual sugar was not increased meaningful together with the reducing of the fermentation time. (We mention that the value of about 2% was obtained in the integral fermentations presented in [6]). This conclusion is distinguished in the fourth column of the table 2:

I : 2.027 $\longrightarrow$ 2.14% (+ 5.58 %)
II : 2.35% $\longrightarrow$ 2.43% (+ 3.48 %)
III: 2.028% $\longrightarrow$ 2.29% (+ 12.92 %)

In conclusion, for the yields mentioned before, the following results were obtained:

- the ethanol yield, $Y_{P/S}^{fin}$ together with the experimental energetic yield, η_{exp}^{fin} increased with 2.49%, from 0.486 to 0.498, and respectively from 95.03% to 97.40%;
- the technological energetic yield, η_{tech}^{fin} increased only with 1.89%, from 79.57% to 81.08%;
- the biggest increase was registered to the medium productivity of the bioprocess, now with 252.61%, from 0.555 g$\cdot kg^{-1} \cdot h^{-1}$ to 1.956 g$\cdot kg^{-1} \cdot h^{-1}$, because the working effective time has been reduced with 69.83% from 92 h and 49.75 min to 28 h and 0.19 min as a result of the bioprocess efficiency.

It is evident that, increasing the average temperature of the bioprocess, with about 12°C (12.14°C), together with the maintenance of the bioreactor mass only in the limits allowed by the economic efficiency, we can obtain an increasing of the average productivity of 3.5 times, without affecting other qualitative parameters of the bioprocess, possible positive.

But, how it should be evaluated a priori the minimum time needed for the fermentation, in certain thermal conditions, starting from a standard process? This is a question that we tried to give an answer through the thermo-kinetic Arrhenius type model.

The activation energy term together with the pre-exponential factor, induced based on the experimental data of the fermentations, which is a temporal efficiency, can give approximately an evaluation of this question.

Thus, on the basis of the parameters presented in this part of the work, we can appreciate the time of an alcoholic fermentation, realised in identical conditions with those from our experiments, at least for the interval of average temperature, respectively 22÷35°C.

But, concurrently, with the purpose to obtain values for the activation energy and for the pre-exponential factor one can restart experiments for other fermentative variants with certain nutritive medium compositions and another criterion employable to close the bioprocess using the methodology presented in these two parts of the paper. The experimental values should be probably close, if the conditions will correspond approximately to those presented in this paper.

Certainly, it can be chosen other graphical or statistical procedure with lower or absent theoretical aspects with the intention to obtain a kinetic model.

Annex

p_{et}^{fin}- total biosynthesized ethanol at the economic end of the bioprocess, g;

S_{fin} - total residual sugar at the economic end of the bioprocess, g;

ΔS^{fin} - total consumed sugar in economical finalised fermentations, g;

$Y_{P/S}^{fin}$ - product yield obtained relative to substrate for economical finalised fermentations, $g \cdot g^{-1}$;

P_{m}^{fin} - global gravimetric productivity for economical finalised fermentations, $g \cdot kg^{-1} \cdot h^{-1}$;

η_{exp}^{fin} - experimental energetic yield for economical finalised fermentations, %;

η_{tech}^{fin} - technological energetic yield for economical finalised fermentations, %;

$[S]^{fin}$-residual sugar concentration at the economic end of the bioprocess, %;

1/T - inverse of the absolute medium temperature for economical finalised fermentations, K^{-1};

k - specific rate constant for economical finalised fermentations, s^{-1}.

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