MODELING OF CONTINUOUS ETHANOL FERMENTATION IN IDEAL MIXING COLUMN BIOREACTOR

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KEYWORDS

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ABSTRACT

A method for formalization and analytical determination of the kinetic parameters of continuous alcohol fermentation in a reactor and in a cascade of reactors with ideal mixing was discussed in the present work. A fluidized bed bioreactor with immobilized yeast with elucidated structure of the flows was used. The method of formalization of the process kinetics involved two steps – determination of the optimal dilution rate of a continuous fermentation process, and determination of the total kinetic parameters of the cascade of reactors, including the minimum number of steps that can ensure production of maximum ethanol yield in the system.

INTRODUCTION

Ethanol is a fermentation product, which is widely used in food and chemical industry and as a bio-fuel. Liquid bio-fuels are divided into the following categories: (a) bio-alcohols; (b) vegetable oils and biodiesels; and (c) bio-crude and bio-synthetic oils. In EU several instruments encouraging bio-fuel and especially ethanol production are approved: The white book "Energy for the future: renewable energy sources" from 1997; The green book "Towards European strategy for energy supplies stability" from 2000; Directive 2003/30/EU for encouragement of bio-fuels and other renewable energy sources utilization in transportation from May 2003 (Berg 2004; Demirbas 2008; Directive 2003/30/EC; Kosaric and Vardar-Sukan 2001; Thomsen et al. 2003; Zaldivar et al. 2001).

Worldwide ethanol is obtained through fermentation of boiled raw cereals (wheat, barley, maize), potato, lignocellulosic materials, bagasse, etc., which are subjected to a fermentation process using yeast of the species *Saccharomyces cerevisiae*. The fermentation process is still a subject to a number of studies regarding the optimization of its parameters - time, temperature, process equipment (Berg 2004; Demirbas 2008; Directive 2003/30/EC; Kosaric and Vardar-Sukan 2001; Thomsen et al. 2003; Zaldivar et al. 2001).

Culturing of microorganisms (alcohol fermentation) occupies an important place in ethanol production. The consumption of sugars by yeast is accompanied by the accumulation of yeast biomass, ethanol and a number of secondary metabolites - esters, aldehydes and higher alcohols. The modelling of the fermentation process, its optimization and intensification are based on the formalization of the process kinetics after a number of assumptions. Usually formalization is done in terms of ethanol accumulation as it is considered to be the only product of yeast biomass metabolism. This formalization is based on one of the two assumptions: microorganisms simultaneously multiply and accumulate product or microorganisms do not multiply, thus acting as a biocatalyst. The second assumption is valid for systems with immobilized cells, in which the localization of cells in the matrix of the carrier limit their growth to a minimum, leading to their action only as a catalyst of the fermentation process (Kostov 2015; Malek and Fencl 1968; Pirt 1975; Willaert et al. 1996; Yarovenko 2002).

The equipment layout, mainly the type of fermentation system used - reactor with ideal mixing, reactor with ideal ejection or a real reactor, is important for the formalization of the alcohol fermentation kinetics. The structure of the flows in the apparatus largely determine the substrate consumption rate and the specific release rate of the target product (Willaert et al. 1996; Levenspiel 1999).

The purpose of the present work was to present an analytical method for the determination of kinetic parameters of a continuous fermentation process in a reactor with ideal mixing. A column bioreactor with immobilized biocatalyst in a fluidized bed, with a known flows structure was selected as a model reactor. In the fulfillment of this purpose two problems were solved – determination of the optimal dilution rate in one fermentation step and determination of certain kinetic parameters in a cascade of reactors with ideal mixing.

MATERIALS AND METHODS

Microorganisms, media and immobilization conditions.

The study was performed with the dry yeast *Saccharomyces cerevisiae* 46EDV supplied by the Company "Martin Vialatte OEnologie", France. Yeasts were stored under refrigeration conditions and were rehydrated according to manufacturer's recommendations prior to the survey.

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For the conduction of the fermentation process was used a medium with pre-optimized qualitative and quantitative composition (g/dm^3): glucose - 118.40; $(NH_4)_2SO_4$ – 2; KH_2PO_4 – 2,72; $MgSO_4x7H_2O$ – 0,5; yeast extract – 1 (Kostov, 2007; Kostov, 2015).

The cells were immobilized in a 4 % calcium alginate gel. After autoclaving the alginate solution for 20 min at 120 °C, the solution was mixed with the cell suspension to obtain a cell concentration of 10^8 cells/mL of gel. This suspension was forced through a syringe needle by means of peristaltic pump and dropped into 2 % (w/v) $CaCl_2$ solution. The resulting beads were approximately 2 mm in diameter. The beads were left for 30 min in calcium solution and then were washed with physiological solution (saline solution) (Kostov 2007).

Bioreactor and culturing conditions

The laboratory bioreactor (Fig. 1) with a fluidized bed "F2" was a glass cylinder (1) with a height of 420 mm, disposed between stainless steel flanges. The apparatus was equipped with a cylindrical phase separator (6) and a liquid degassing cylinder (10) with a height of 100 mm. The column was provided with a cover on which the electrodes for monitoring the fermentation process were placed.

The temperature of the culture medium was measured by RTD (16) and was controlled by the control unit by switching on the heater (13) and periodically passing the cooling water in the heat exchanger (17) through a magnetic valve (15).

The pH during fermentation was kept constant at the optimum value of pH=4.5 using 10% H_2SO_4 and 20% KOH with the help of peristaltic pumps, operated by the control unit. The pH was measured using a combined electrode (12).

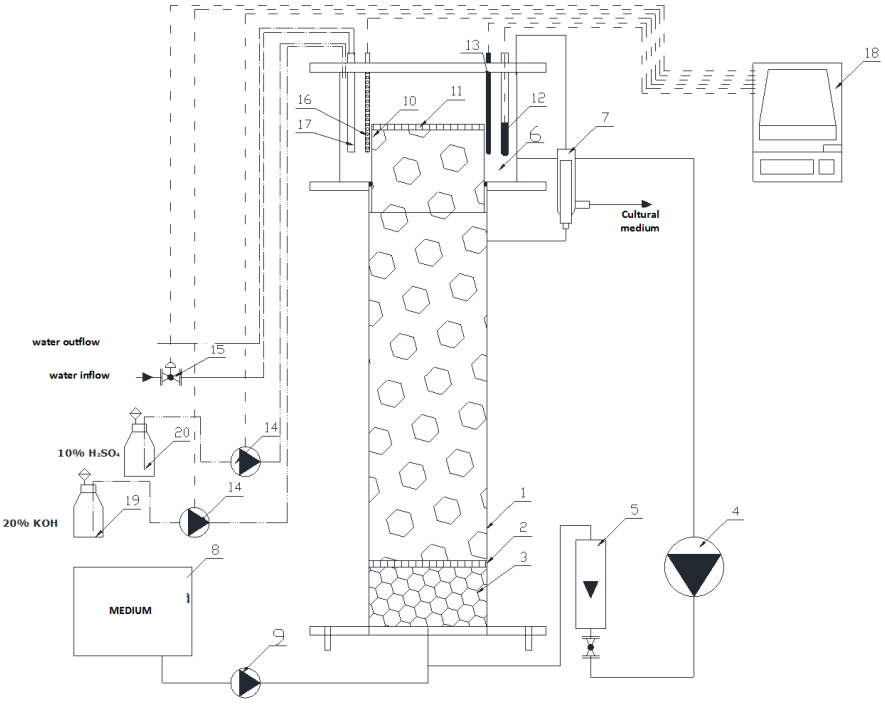

Figure 1: Scheme of a Laboratory Bioreactor With a Fluidized Bed

1 - column; 2 – grid; 3 - drainage; 4 - peristaltic pump; 5 - calibrated rotameter; 6 - phase separator; 7 - outgoing cell; 8 – medium reservoir; 9 - peristaltic pump for continuous operation; 10 – gas separator; 11 – grid; 12 - pH electrode; 13 - heater; 14 - pumps for pH correction; 15 - solenoid valve; 16 - RTD Pt 100; 17 - heat exchanger for submission of cold water; 18 - control device "Applikon"; 19,20 - banks for pH – reagents

300 g of the immobilized preparation and 1,8 dm^3 of the broth medium were placed in the apparatus. The preparation was brought in a fluidised state by means of a pump (4) and batch fermentation at 28 °C for 24 h, which provided ethanol yield of 50% in the reactor, was conducted after the establishment of a steady state of fluidization. The system was then put into a continuous mode, by turning peristaltic pump (9) on. The flow rate of the supplied fluid was determined by the required dilution rate. Constant fluid volume was maintained through spillway (7).

CO_2 formed during fermentation left the apparatus through a heat exchanger placed on the lid of the column in which cold cooling water was circulating. The alcohol vapors escaping from the apparatus were being condensed in the heat exchanger.

Analyses

The concentrations of ethanol and glucose in the medium were determined automatically using an Anton Paar DMA 4500 ", Austria.

The biomass concentration in the immobilized preparation was determined after its dissolution in

sodium citrate according to the cell-free spectrophotometrical method at a wavelength of 620 nm (Zhou et. al. 1998).

RESULTS AND DISCUSSION

Structure of the flows in the reactor

The structure of the flows in the bioreactor was defined in a series of studies. From the analysis of the tracer concentration at the output of the system, it can be determined that the bioreactor with immobilized cells in a fluidized bed and recirculation of the culture medium may be referred to the group of reactors with ideal ejection (Fig. 2) (Kostov 2007).

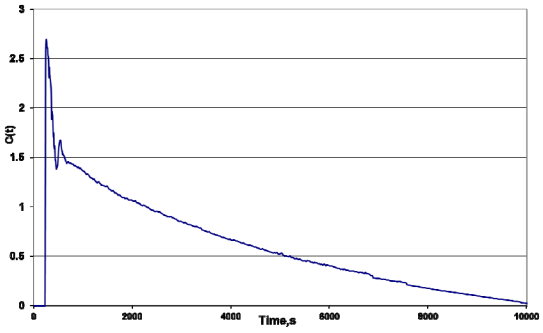

Figure 2: Changes in the Tracer Concentration at the Output of the System

Kinetics of continuous fermentation process in one fermentation step in a reactor with ideal mixing

A series of experiments to determine the optimal dilution rate of the fermentation process (D, h^{-1}) were conducted. The results are presented in Fig. 3 and Fig. 4. The optimum dilution rate was based on the system productivity, which was presented as the multiplication the product quantity at the output of the system and the dilution rate - Q = P.D, g / (dm^3.h). The concentration of the basic fermentation process parameters was determined as the average concentration at the system output at time corresponding to more than 3/D.

Unlike the preparation of biomass in continuous systems where D=μ, the specific rate of product formation was also influenced by the concentration of biomass and product in the medium. Thus, various combinations and relative freedom exist in choosing the appropriate conditions for continuous process in this case. The microorganism concentration itself did not uniquely determine product formation. It was only one of the variables that were crucial to the process, namely representing the total amount of active enzyme systems involved in the reaction. This amount varied depending on the physiological state of the culture, and was generally determined by the age, the culture conditions, etc. (Malek and Fencl 1968).

Another important condition for the development of the continuous fermentation model was the assumption that the process with immobilized cells could be described by the equations for free cells. This assumption was valid at zero and minimal impact of the internal diffusion resistances. Similar negligible influence was proven in the work of Kostov, 2007. Under these conditions, the fermentation process was described by the following system of differential equations:

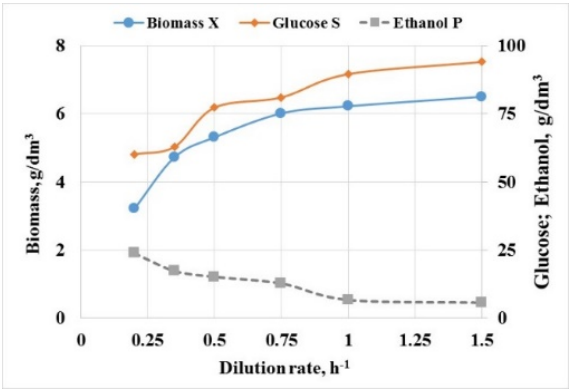

Figure 3: Changes in the Fermentation Process Parameters at Various Dilution Rates

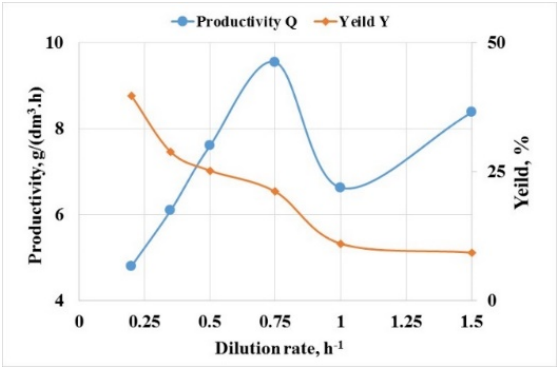

Figure 4: Changes in the Productivity of the System and the Ethanol Yield at Various Dilution Rates

$$\begin{cases} \dfrac{dX}{d\tau} = \mu X - DX \\ \dfrac{dP}{d\tau} = q_p X - D.P \\ \dfrac{dS}{d\tau} = D(S_0 - S) - \dfrac{q_p X}{Y_{P/S}} \end{cases} \quad (1)$$

wherein: X, S, P were the concentrations at the outlet of the system of biomass, substrate and product, g/dm^3; μ - specific growth rate of the biomass, h^{-1}; q_p - specific rate of ethanol accumulation, g/(dm^3.h); D - dilution rate, h^{-1}; $Y_{P/S}$ - product yield per unit substrate.

It is characteristic of the immobilized cell system that the biomass is confined within the matrix of the carrier and does not leave the volume of the apparatus. Assuming that condition, dX/dτ=0, but since all calculations of the kinetic characteristics included biomass growth, it was assumed that its concentration inside the carrier was determined by the first equation in the equation system (1).

The maximum biomass yield and the specific expense of the substrate to maintain the vital activity of the microorganisms were linked by the relationship:

$$q_S = \frac{D}{Y_{X/S}} = \frac{D}{Y^m_{X/S}} + m_S = aD + b \qquad (2)$$

wherein: $a=1/Y^m_{X/S}$ and $b=m_S$.

In order to determine the values of the parameters in equation (2), an equation to describe the specific growth rate in stationary continuous mode should be defined. In the absence of substrate inhibition it could be assumed that:

$$\mu = \mu_m \frac{S}{K_S + S} - m_S Y^m_{X/S} \qquad (3)$$

This equation is also known as Monod model for hemostat culturing (Pirt, 1975).

The relation between q_S and D could be determined at stationary mode at $dP/d\tau=0$ and $dS/d\tau=0$:

$$q_S = \frac{D}{\frac{Q}{q_{p0}(S_0 - S)}} \qquad (4)$$

The function $q_S(D)$ was plotted using equation (4) and the parameters in equation (2) were defined (Fig. 5). The parameter q_{p0} represented the specific rate of product accumulation in batch mode (Kostov, 2007). It had a value of $q_{p0} = 2.052$ g/(dm^3.h).

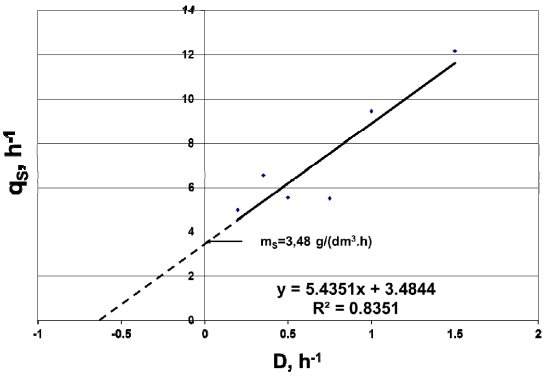

Figure 5: Graphical Definition of the Parameters in Equation (2)

The coefficient m_S=3,48 g/(dm^3.h) was equal to the specific consumption of substrate for maintenance of the vital activity of existing cells at a given time. It characterized the physiological state of the culture. This part of the total amount of consumed substrate was consumed in the synthesis of cellular structures, for maintaining ionic gradients and neutral molecules between the cell and the medium, and between the different structures of the cell (Pirt, 1979). The high value of m_S was most likely due to the large amount of viable cells in the volume of the medium and to the reduced living space in the pores of the carrier.

After the determination of the parameters in equation (2), the values of the constants in equation (3) were determined using the method of least squares. From experimental data and the adoption of stationary regime of the system the kinetic constants were μ_m = 1,49 h^{-1}; K_S=9,113 g/dm^3; $Y^m_{X/S}$=0,184 g/g (R^2 = 91,68%). Data showed a relatively high specific growth rate, due to the high volume of viable biomass in the reactor and the fact that the biomass grew without leaving the apparatus. $K_S << S$, which indicated high affinity of the biomass to the substrate. The value of the constant K_S was much smaller than the values listed in Rovinski and Yarovenko, 1978, where the value was commensurable with the concentration of substrate in the medium.

The other parameters of the system of differential equations (1) could easily be determined from the stationary regime of the system:

$$\tilde{P} = q_p \frac{\tilde{X}}{D} \qquad (5)$$

$$\tilde{S} = S_0 - \frac{q_p \tilde{X}}{Y^m_{P/S} D} \qquad (6)$$

Equations (5) and (6) give the relationship between the kinetics of the fermentation process and the steady state of the system. The parameters in them can be identified by experimental data of the fermentation system, but only if it is in stationary mode.

By experimental data from Fig. 3 and Fig. 4 using the method of least squares it was found out that q_P = 2,047 g/(g.h), $Y^m_{P/S}$ = 0,298 g/g. The correlation coefficient between experimental data and the models ranged from 74.5% to 84.4%. The model for the description of product accumulation underestimated experimental data at low dilution rates, but fairly accurately described the experimental values at medium and high values of D, i.e. within the operating range of the system. The specific rate of product accumulation was close in value to that of the batch process, which confirmed the accepted assumption that the constants could be calculated by the batch process.

A system of explicit equations for the stationary regime was obtained as a result of the analytical identification conducted:

$$\begin{cases} \tilde{X} = 0{,}382.D.\left(\dfrac{118{,}4}{D+3{,}48} - \dfrac{9{,}113}{1{,}49-D}\right) \\ \tilde{P} = 0{,}782.\left(\dfrac{118{,}4}{D+3{,}48} - \dfrac{9{,}113}{1{,}49-D}\right) \\ \tilde{S} = 118{,}4 - 2{,}322.\left(\dfrac{118{,}4}{D+3{,}48} - \dfrac{9{,}113}{1{,}49-D}\right) \\ \tilde{Q} = 0{,}782.D\left(\dfrac{118{,}4}{D+3{,}48} - \dfrac{9{,}113}{1{,}49-D}\right) \end{cases} \qquad (7)$$

The fourth equation in the system (7) represented the functional dependence of system productivity in stationary regime. The results for the fermentation process dynamics showed that the system productivity had its maximum in a relatively narrow area of dilution rates - about 0,7-0,8 h^{-1}. The product concentration,

therefore its yield, decreased with increasing the dilution rate, which was related to the lack of sufficient time for stay of the fluid in the fermentation area and therefore insufficient time to conduct the biological transformation. Through numerical optimization of the fourth equation in system (7), the optimal value of the dilution rate could be determined (Table 1).

Table 1: Parameters of the System at the Optimal Dilution Rate

D_m	$\tilde{X}_m$	$\tilde{P}$	$\tilde{S}$	$\tilde{Q}_m$,
h^{-1}	g/dm^3			g/(dm^3.h)
0,725	4,52	12,76	80,72	9,265

Kinetics of continuous fermentation process in a cascade of reactors with ideal mixing

Data in Table 1 showed very low product yield (about 21%) and poor sugars comsuption. This required that the fermentation was carried out in several consecutive steps (a cascade of reactors with ideal mixing). A simulation study was conducted and the parameters of the fermentation process in successive fermentation steps were defined based on the kinetics in one step under the following assumptions (Table 2): the dilution rate was determined by the dilution rate in the first step; the output parameters of a given apparatus formed the input parameters of the next apparatus; the kinetics in successively connected apparatuses was described by the same equations; the biomass concentration was a constant value for each one of the fermentation steps.

Data in Table 2 showed that the system productivity in the last two apparatuses of the cascade was from 1.5 to 3 times lower than that in the first 3 apparatuses. This can be easily explained, after the determination of the kinetic parameters of the cascade.

The following system of differential equations was used for description of the kinetics of the processes in the cascade of apparatuses (Rovinski and Yarovenko, 1978):

$$\begin{cases} \dfrac{dX_i}{d\tau} = D_i(X_{i-1} - X_i) + \mu_i X_i \\ \dfrac{dP}{d\tau} = D_i(P_{i-1} - P_i) + q_{pi}\mu_i X_i \\ \dfrac{dS}{d\tau} = D_i\left(S_{i-1} - S_i\right) - \dfrac{\mu_i X_i}{Y} - \dfrac{k_i P_i S_i}{K_{Mi} + S_i} \end{cases} \quad (8)$$

wherein: X_i, S_i, P_i were the concentration at the outlet of the system of biomass, substrate and product in the i-th apparatus, g/dm^3; μ_i -specific growth rate of the biomass in the i-th apparatus, h^{-1}; q_{pi} - specific ethanol production rate in the i-th apparatus, g/(dm^3.h); K_{Mi} - saturation constants of the metabolic products in the i-th apparatus; k_i - kinetic constant/coefficient of the reaction rate in the i-th apparatus;

In the third equation of the differential equation system compared with the system (1), a correction that shows growth inhibition in the cascade of apparatuses after the first one due to ethanol accumulation should be made. This adjustment is made by the member:

$$\frac{k_i P_i S_i}{K_{Mi} + S_i}.$$

The following dependencies were valid in stationary regime of the system (Rovinski and Yarovenko, 1978):

$$\begin{aligned} X_i &= \frac{D_i X_i - 1}{D_i - \mu i}; \\ P_i &= P_{i-1} + q_{pi}(X_i - X_{i-1}) \end{aligned} \quad (9)$$

The Monod equation was used for the description of the specific growth rate:

$$\mu_i = \mu_{mi}\frac{S_i}{K_S + S_i} \quad (10)$$

Table 2: Fermentation Process Parameters in a System of Consequtively Connected Apparatuses with Ideal Mixing

№ of apparatus	D_m	$\tilde{X}_m$	$\tilde{P}$	$\tilde{S}$	$\tilde{Q}_m$ *
	h^{-1}	g/dm^3			g/(dm^3.h)
1	0,725	4,52	12.76	80.72	9.27
2			25.61	55.6	9.31
3			36.55	34.2	7.93
4			44.84	18	6.01
5			49.95	8	3.71

* calculated based on the ethanol produced in the step

The system of equations (8) can be solved analytically if taking into account the already described assumptions as well as equations (9) and (10). The methodology for solving the system of equations was presented in Rovinski and Yarovenko, 1978 and the following relationships were obtained as a result of its implementation:

$$\begin{aligned} y_i &= b_0 + b_1 x_i \\ y_i &= \frac{Y(\alpha_T - \alpha'_{i-1})}{\alpha'_{i-1} - \alpha_T(1-Y)};\ \mathrm{x_i} = \frac{1}{i}; \\ b_0 &= \frac{\mu_m K_M}{k q_p K_S};\ b_1 = \frac{D(K_S - K_M)}{k q_p K_S} \end{aligned} \quad (11)$$

wherein: b_0 and b_1 - coefficients; Y - economic coefficient (biomass yield); α', α_T - real and theoretical ethanol yield (α' - the yield was calculated for each step based on the sugars utilized in the step; α_T = 0,5114 - calculated based on the stoichiometry of the alcohol fermentation process at 20 °C (Yarovenko and Rovinski, 1978)); μ_m - maximum specific growth rate of the biomass, h^{-1}; q_p - specific ethanol production rate in one fermentation step or in a cascade of apparatuses, g/(dm^3.h); K_S, K_M - constants of saturation of the substrate and the metabolic products; k - kinetic constant/coefficient of the reaction rate;

The coefficients b_0 and b_1 could be determined by the fermentation process dynamics. The kinetic parameters in the cascade of reactors with ideal mixing could be defined using the coefficients b_0 and b_1. To carry out the

calculations all kinetic parameters were assumed to be equal to the kinetic parameters calculated in the first step.

To determine the kinetic parameters in the equations (11) it was necessary to determine the value of y_i in each of the steps. Thus, the actual ethanol production, which is the ratio between the amounts of product accumulated in the step and the theoretical amount of ethanol, which would be the result of full utilization of the substrate entering the given apparatus, was calculated. After the determination of the parameter y_i, $y_i = f(x_i)$ was plotted, which was compared with the equation of a straight line (Fig. 6). The calculations after the first apparatus were made at a constant dilution rate according to the methodology of calculating (Rovinski and Yarovenko, 1978). The parameters of the equation of the straight line were the coefficients b_0 and b_1.

The kinetic constants for the cascade of reactors with ideal stirring were calculated based on the graphical determination of the parameters in equation (11): kq_P = 11.67 g/kg and K_M=12.03 g/dm^3. These two constants were the averages for the entire cascade of apparatuses with ideal mixing.

A major disadvantage of this method was that the whole cascade of apparatuses was formalized to one apparatus with ideal mixing.

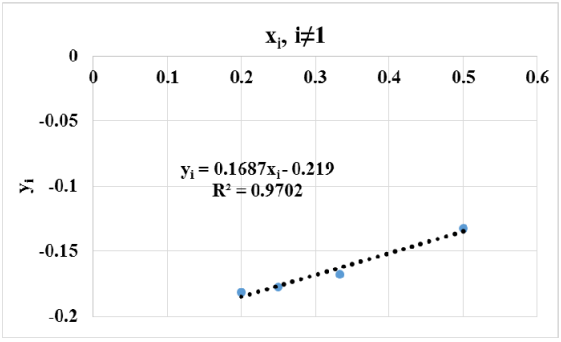

Figure 6: Graphical Definition of the Parameters in Equation (11)

However, the use of the method allowed to determine some important system parameters. Firstly, the minimum number of apparatuses, which was necessary to effect complete transformation of the substrate was 3 (Table 2). Secondly, the estimation of K_M indicated the presence of product inhibition, which was particularly strong in the last two apparatuses of the cascade (apparatus 4 and 5).

Data from Table 2 and the identified kinetic parameters indicated that fermentation could be completed in 3 apparatuses. This could be done by two approaches – by optimization of the dilution rate of each apparatus and by optimization of the fluidisation conditions, that is, by reduction of the impact of the so-called external diffusion resistances in the system. These two optimization problems themselves are interesting and will be the subject of subsequent publications. However, the kinetic parameters as shown in the present publication need to be determined in order these new problems to be solved.

Another important feature of the system in question is the fact that there was a significant amount of ethanol in the capsules, which was supposed to leave them by diffusion. For this purpose it is also necessary to optimize the hydrodynamic environment in the apparatus, but it must not distort the structure of flows in it.

The comparison of the results in Table 2 with data of real experiment is the subject of study in the present work. Initial results showed that in the second stage productivity of about 9 g/dm3 was achieved upon reaching ethanol yield of about 75% of the theoretical yield (total yield in two fermentation steps). This is encouraging, and is due to the fact that the fermentation is carried out in optimized hydrodynamic conditions that are also object of the present study.

The results obtained are comparable with the data presented in Rovinski and Yarovenko, 1978, differing only in the the dilution rate that was several times higher in value. The high value of the dilution rate established was due to the increased concentration of cells in the volume of the apparatus, which was one of the advantages of immobilized cell systems.

CONCLUSION

The present publication presents an analytical approach for determination of part of the kinetic parameters of alcohol fermentation process performed in a single apparatus or a cascade of apparatuses with ideal mixing. The kinetic parameters were defined graphically and analytically based on experimental data on the dynamics of the fermentation process with immobilized cells in a fluidised bed reactor. They were used to determine by simulation the dynamics in the cascade of fermentation apparatuses, as well as for the determination of the minimum number of steps in the cascade, and its kinetic characteristics.

A great advantage of the proposed method is that it eliminates to a great extent the specific hydrodynamic dependencies in the apparatus (especially fluidized bed reactors) and formalizes relationships to systems with ideal mixing. Thus, if it is possible to prove that an apparatus is with ideal mixing, the proposed methodology can be applied to the apparatus and to a cascade of similar apparatuses. An important condition for the application of the methodology is the identification of kinetic parameters in the apparatus to be done in a stationary mode, otherwise kinetics will be linked to the specific type of bioreactor used. It is important to note that the fermentation kinetics will be affected by the type of the selected fermentation - with free or immobilized cells, which would change the specific representation of the differential equation systems (1) and (8).

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