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Kinetic Study of *Saccharomyces pastorianus (carlsbergensis)* Multiplication

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Abstract. In this paper we derive an approximating model for the kinetic reaction of the processes in yeast multiplication. The model comprising a Michaelis-Menten mechanism for yeast's feeding and other two elementary reactions for multiplication and degradation of the yeasts were qualitatively compared with a series of experimental results for yeast fermentation. The analysis given an estimate for the minimum number of experiments required to obtain good estimates for the parameters of the yeast fermentation process.

Keywords*:* kinetics, Michaelis-Menten reaction, multiplication, *Saccharomyces pastorianus (carlsbergensis)*

INTRODUCTION

Henry (1903) has discover that enzymatic reactions has take place because of connection form between enzyme (E) and substrate (S), but the action of the enzymatic mechanism it's observed for the first time on Invertase efficiency kinetics study by Michaelis and Menten (1913). These have start from the fallowing premise: enzyme concentration is negligible in comparison with substrate concentration but initial velocity reaction and concentration changes of the product (P) or substrate (S) are not significant.

$$S + E \leftrightarrow C \rightarrow P + E$$

where: S - substrate, E - enzyme, C - complex, P - product (concentration: s, e, c, p).

In the first phase of reaction enzyme and substrate gives the enzyme-substrate complex (C). This stage is quick and reversible. On the second phase enzyme-substrate complex (C) dissociate in product (P) and is relishing the enzyme (E) does not change the total concentration over time.

Obtaining kinetic model allows the representation theory of evolution to the equilibrium system, involves writing equations velocity of all elementary reactions and the principle of conservation of mass.

Solving of the Michaelis-Menten mechanism suppose following reasoning:

÷ Writing of the elementary reactions:

(1): $S + E \rightarrow_{k1} C$, $v_{(1)} = k_1 \cdot s \cdot e$;
(2): $C \rightarrow_{k2} S + E$, $v_{(2)} = k_2 \cdot c$;
(3): $C \rightarrow_{k3} P + E$, $v_{(3)} = k_3 \cdot c$;

÷ Writing of the mass conservation principle:

(S): $\dot{s} = v_{(2)} - v_{(1)}$

(E): $\dot{e} = v_{(2)} + v_{(3)} - v_{(1)}$

(C): $\dot{c} = v_{(1)} - v_{(2)} - v_{(3)}$

(P): $\dot{p} = v_{(3)}$

÷ Making some premises such as:
  - $s(0) = s_0$;
  - $e(0) = e_0$;
  - $c(0) = 0$;
  - $p(0) = 0$;
  - $e = e_0 - c$;

÷ Obtaining of the equations to solve:
  - $\dot{s} = k_2 c - k_1 s(e_0 - c)$
  - $\dot{c} = k_1 s(e_0 - c) - (k_2 + k_3)c$

÷ At this point are two possible approximations:
  - $\dot{c} = 0 \Rightarrow c = \frac{e_0 s}{\kappa + s};\ -\dot{s} = \dot{p} = \frac{k_3 e_0 s}{\kappa + s};\ \kappa = \frac{k_2 + k_3}{k_1}$ (Briggs & Haldane, 1925)
  - $\dot{s} = 0 \Rightarrow c = \frac{e_0 s}{\kappa + s};\ \dot{p} = \frac{k_3 e_0 s}{\kappa + s};\ \kappa = \frac{k_2}{k_1}$ (Henry, 1903)

Kinetics of enzymatic reactions with one substrate is based on standing-state theory developed by Briggs and Haldane (1925) which assumed that during the progress of enzymatic reaction enzyme-substrate complex concentration remains constant. In this way, the rate of formation of C complex is equal to the rate of consumption. In same manner, Henry (Henry, 1903) assumed that the substrate is quite enough ro be considered constant.

Anyway, with proper substitutions:

$$a = \frac{k_2}{k_2 + k_3};\ b = \frac{k_2 + k_3}{k_1 e_0};\ x = \frac{k_1 s}{k_2 + k_3};\ y = \frac{c}{e_0};\ t = k_1 e_0 \tau$$ (τ is the initial time variable)

the general case leads to the explicit equation:

$$\dot{x} = -x + ay + xy;\ \dot{y} = b(x - y - xy);\ 0 < a < 1;\ b > 0$$

and to an implicit equation (in the phase space):

$$\frac{dy}{dx} = b\frac{x - y - xy}{-x + ay + xy}$$

Unfortunately the problem has no analytical solution and is the main reason for which different approximations were proposed.

Fortunately, numerical solutions can be obtained starting from the explicit equations.

Chemicals such as ammonium sulphate can have a stimulating effect, beneficial effect on yeast cells at low concentrations, because they contain elements that go into cellular compounds. At higher concentrations of chemicals may affect growth stagnation or even fatal (Dumitru & others, 2002). Yeasts are able to synthesize nitrogen as the main element in plastic, from both organic and inorganic compounds, nitrogenous substance use is strongly influenced by the degree of aeration of the worth (Oprean, 2002). The brewer's yeast biotechnological qualities depend on their metabolic state, condition of growth media and on their ability to adapt to different stress condition (Gervais and Martinez de Maranon, 1995).

Starting from these considerations and having a series of experimental results concerning growth of the yeast in different conditions in this paper we have attempted to model the reaction kinetics of multiplication of yeast in different conditions of temperature and substrate. The Michaelis-Menten mechanism was used to model feeding and other two reactions were used for multiplication and degradation respectively.

MATERIALS AND METHODS

Experimental measurements setup. Strains (originated from *Saccharomyces carlsbergensis*) of Weihenstephan 34/70 (SAFLAGER WB 34/70 from Crosby & Baker) was propagated and further used in a yeast growth study on malt extract broth (Art. No. 02-491 from Scharlau Chemie) at two different temperatures and $(NH_4)_2SO_4$ added salt (Tab. 1).

Yeast and growth medium (containing 17g/l malt extract and 3g/l peptone) was inserted into a BIOSTAT® A plus 5 L (from Sartorius AG) autoclavable bioreactor and the optical density were recorded at four moments using a spectrophotometer at 600 nm (Tab. 1) and automatically converted into biomass concentration units (BioPAT® MFCS/DA data storage and plotting software package).

Tab. 1

Factors in the experimental design for yeast concentration measurements

Factor 1	Factor 2	Factor 3
Temperature (°C)	$[(NH_4)_2SO_4]$ (g/l)	Time (h)
12	0	0
22	0.1	4
	0.5	8
	1.0	12
		24

Kinetic modelling. Three processes were modelled in order to explain the progression of yeast multiplication (Tab. 2).

Tab. 2

Processes in yeast multiplication

Feeding	Multiplication	Degradation
$R_1 + R_2 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} R_1R_2 \xrightarrow{k_3} R_1 + R_3$	$R_1 + R_2 \xrightarrow{k_5} 2R_1 + R_2$	$R_1 \xrightarrow{k_4} R_4$
R_1 - yeast; R_2 - substrate; R_3 - waste products; R_4 - degradation products		

Decomposition of the model from *Table 2* into the elementary reactions is given in *Table 3*.

Tab. 3

The kinetics of the processes in yeast multiplication

Elementary reaction	Velocity equation
$R_1 + R_2 \xrightarrow{k1} R_1R_2$	$v_1 = k_1[R_1][R_2]$
$R_1R_2 \xrightarrow{k2} R_1 + R_2$	$v_2 = k_2[R_1R_2]$
$R_1R_2 \xrightarrow{k3} R_1 + R_3$	$v_3 = k_3[R_1R_2]$
$R_1 \xrightarrow{k4} R_4$	$v_4 = k_4[R_1]$
$R_1 + R_2 \xrightarrow{k5} 2R_1 + R_2$	$v_5 = k_5[R_1][R_2]$

Specie	Mass conservation equation
R_1	$\Delta[R_1] = -v_1(\Delta t) + v_2(\Delta t) + v_3(\Delta t) - v_4(\Delta t) + v_5(\Delta t)$
R_2	$\Delta[R_2] = -v_1(\Delta t) + v_2(\Delta t)$
R_3	$\Delta[R_3] = v_3(\Delta t)$
R_4	$\Delta[R_4] = v_4(\Delta t)$
R_1R_2	$\Delta[R_1R_2] = v_1(\Delta t) - v_2(\Delta t) - v_3(\Delta t)$

In order to simulate the processes of yeast multiplication, following notations were used for variables and constants (Tab. 4).

Tab. 4

Constants and variables in the kinetics of the yeast multiplication processes

Initial values	$[R_1]_0 = u0$	$[R_2]_0 = w0$	$[R_3]_0 = 0$	$[R_4]_0 = 0$	$[R_1R_2]_0 = z0$
Variables	$[R_1] = u$	$[R_2] = w$	$[R_3] = x$	$[R_4] = y$	$[R_1R_2] = z$

The equations from *Table 3* were used to construct the differential equations and the finite differences equations of the yeast multiplication processes and are given in *Table 5*.

Tab. 5

Differentials and the finite differences equations in the kinetics of the yeast multiplication

Differential equations	Finite differences equations
$\begin{cases} \dot{u} = -k1 \cdot u \cdot w + k2 \cdot z + k3 \cdot z - k4 \cdot u + k5 \cdot u \cdot w \\ \dot{w} = -k1 \cdot u \cdot w + k2 \cdot z \\ \dot{x} = k3 \cdot z \\ \dot{y} = k4 \cdot u \\ \dot{z} = k1 \cdot u \cdot w - k2 \cdot z \end{cases}$	$\begin{cases} u_i = u_{i-1} - k1 \cdot u_{i-1} \cdot w_{i-1} + k2 \cdot z_{i-1} + k3 \cdot z_{i-1} - k4 \cdot u_{i-1} + k5 \cdot u_{i-1} \cdot w_{i-1} \\ w_i = w_{i-1} - k1 \cdot u_{i-1} \cdot w_{i-1} + k2 \cdot z_{i-1} \\ x_i = x_{i-1} + k3 \cdot z_{i-1} \\ y_i = y_{i-1} + k4 \cdot u_{i-1} \\ z_i = z_{i-1} + k1 \cdot u_{i-1} \cdot w_{i-1} - k2 \cdot z_{i-1} - k3 \cdot z_{i-1} \end{cases}$

RESULTS AND DISCUSSION

The following experimental results were obtained for yeast concentration under the experimental design given in *Table 1* (Tab. 6).

Tab. 6

Observed yeast concentration under different environmental conditions at different moments

F1	F2	F3	[Yst]	F1	F2	F3	[Yst]	F1	F2	F3	[Yst]	F1	F2	F3	[Yst]
Experiment 1				Experiment 2				Experiment 3				Experiment 4			
12	0	0	20	12	0.1	0	20	12	0.5	0	20	12	1	0	20
12	0	4	27.65	12	0.1	4	28.95	12	0.5	4	29.94	12	1	4	29.86
12	0	8	39.27	12	0.1	8	41.45	12	0.5	8	43.65	12	1	8	43.63
12	0	12	47.53	12	0.1	12	49.63	12	0.5	12	49.64	12	1	12	50.55
12	0	24	46.74	12	0.1	24	49.96	12	0.5	24	49.9	12	1	24	49.37
Experiment 5				Experiment 6				Experiment 7				Experiment 8			
22	0	0	20	22	0.1	0	20	22	0.5	0	20	22	1	0	20
22	0	4	37.98	22	0.1	4	38.97	22	0.5	4	39.84	22	1	4	39.77
22	0	8	49.54	22	0.1	8	51.63	22	0.5	8	53.35	22	1	8	53.06
22	0	12	57.06	22	0.1	12	59.58	22	0.5	12	59.95	22	1	12	60.93
22	0	24	56.94	22	0.1	24	59.96	22	0.5	24	59.07	22	1	24	59.99
F1: temperature (°C); F2: concentration of $(NH_4)_2SO_4$ (g/l); F3: reaction time (hours); [Yst]: yeast concentration (g/l)															

Different values for initial values of concentrations, for values of velocity constants and of time step (Δt) were used to give a closest approximation (as good as possible agreement) between observed shape of the yeast concentration and the estimated concentration of yeast - the value expressed as sum between the concentration of un-feed yeast cells (u) and feed ones (z).

The result of best approximating model and observed concentrations are given in *Figures 1* and *2* respectively. The model from *Figure 1* was obtained with the values of the parameters given in *Table 7.*

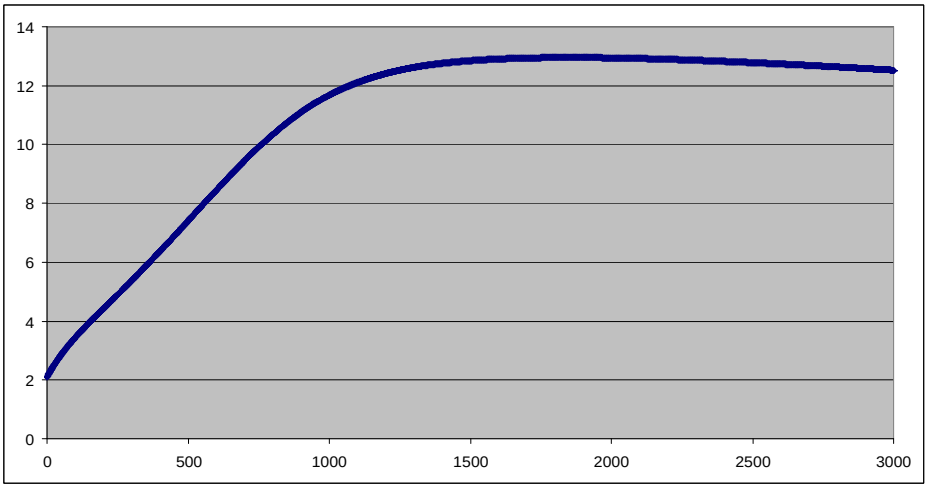

Fig. 1. Yeast concentration (u+z) as function of time - both in arbitrary units

Tab. 7

The parameter values for the model given in Figure 1

Parameter	u0	w0	x0	y0	z0	k1	k2	k3	k4	k5	Δt
Value	2	15	0	0	0.1	0.01	0.006	0.02	0.0005	0.006	0.1

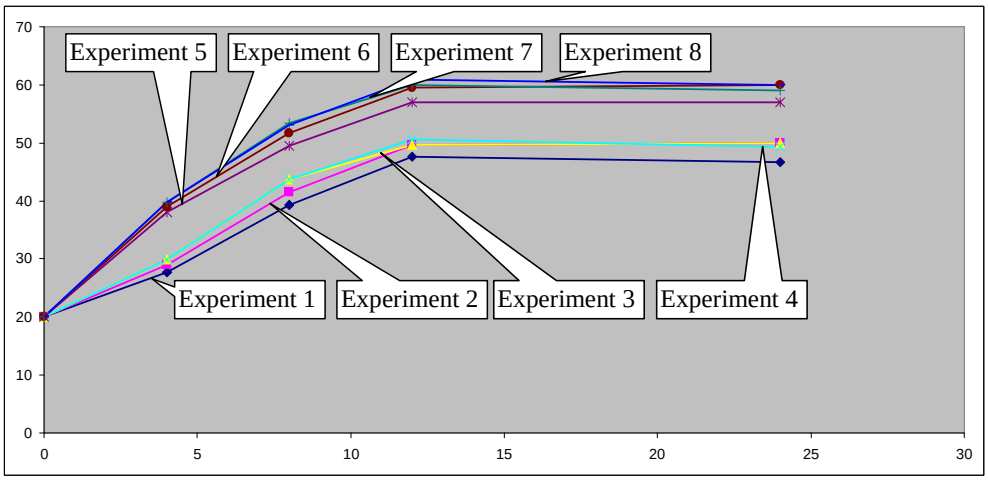

Fig. 2. Observed yeast concentration (u+z) - in g/l - as function of time - in h

The main shape properties of the observed values from *Figure 2* are present in the model from *Figure 1*. Thus, the growth and multiplication process are after a period suppressed due to the decreasing of the substrate concentration, when the concentration of the yeast starts to decrease (about 12h in the experiment; about 1500 time arbitrary units in the model. Anyway, the degradation process creates substrate for the living yeast too, and thus the decreasing of the yeast concentration is relatively (to the increasing phase) slow; the experiment (Fig. 2) and the model (Fig. 1) revealed a good agreement in this phase too.

The values of the velocity constants provided by the model should be in agreement with the true values of the elementary reactions governing the yeast fermentation and it opens

a path to obtain these values for specific yeasts, substrates and experimental conditions. In order to do this, other series of experiments should be conducted with a better-quality division of the timeframe, in order to surpass the greater number of unknowns with a larger number of observed values for the concentration of the yeast during the fermentation process.

CONCLUSIONS

The present study given for yeast fermentation a qualitative comparison of its observed kinetic with a model of fermentation derived from Mihaelis-Menten mechanism, at which multiplication and degradation processes were modelled through elementary reactions. Different ratios between parameter values were tried till the shape of the model tended very well with the shape from experiments. The study it shown that is possible to give very good estimates of the kinetic constants for yeast fermentation using the proposed model of the fermentation process. The obtaining of the accurate values of the kinetic constants requires at least six times more observations than the number of the unknown parameters in the model. The proposed model uses five explicit (the constants of velocities) and one implicit (the time arbitrary unit), and thus 36 observations should be enough for good estimates of the yeast fermentation kinetic parameters.

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