MODELING OF ALCOHOL FERMENTATION IN BREWING – SOME PRACTICAL APPROACHES

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KEYWORD

Brewing, fermentation, immobilized cells, modeling, fermentation kinetics

ABSTRACT

In the present work, a practical method for determination of the basic physicochemical parameters of beer - real extract, alcohol and biomass concentration based on the amount of produced CO_2 during the fermentation is investigated. The method was applied for determination of biomass concentration in immobilized preparations after its approbation with analytical data for beer fermentations with free cells.
The kinetics parameters of the fermentation were determined with 3 of the most used kinetic models. The differences between beer fermentations with free and immobilized cells were investigated. The effect of yeasts immobilization on brewing process was defined.

INTRODUCTION

The beer is made from malt, hops, yeast and water. Non-malting adjuncts such as barley, rice, corn meal, wheat and others are commonly used. They reduce the cost of the product; improve the wort extract and beer flavor and foam. The main stages in the brewing process are: wort production, alcoholic fermentation and maturation, processing and stabilization of the beer (Kunze 2003; Handbook of brewing: Processes, Technology, Markets 2009).
The wort transforms into beer during alcoholic fermentation and maturation, which are the longest processes in brewing. The primary fermentation lasts between 3-6 days and the maturation - up to 2 weeks depending on the fermentation type and the used equipment. The ethanol fermentation occurs as a result of enzymatic activity of the yeast at Embden-Meyerhof-Parnas pathway, which leads to glucose conversion to pyruvate. Under anaerobic conditions the yeasts convert pyruvate to ethanol and CO_2. In aerobic conditions, yeasts consume sugars, mainly for biomass accumulation and CO_2 production (Boulton and Quain, 2001).
Yeasts uptake the carbohydrates of wort in a specific sequence: monosaccharides (glucose and fructose), disaccharides (sucrose and maltose) and trisaccharide maltotriose and ferment them in the same order. Very small amount of maltotriose is used for the formation of reserve polysaccharides (glycogen and trehalose). The amino acids assimilated by yeast are used for the synthesis of proteins, enzymes and new cells. The fermentation by–products: carbonyl compounds, higher alcohols, esters, organic acids and sulfur-containing compounds determine the flavor profile of beer and affect on beer quality. The following processes are carried out during maturation: fermentation of the remaining fermentable extract, saturation with CO_2, removal of unwanted aroma compounds, excretion of flavor-active compounds from yeast to give body and depth to the beer, sedimentation of yeast cells (Kunze 2003; Willaert 2007).
The amount of released CO_2 during fermentation is a direct indicator of fermentation activity of yeast. The ethanol fermentation can be described by the following stoichiometric equation:

$$\underset{1kg}{C_6H_{12}O_6} \xrightarrow{yeast} \underset{0,51kg}{2C_2H_5OH} + \underset{0,49kg}{2CO_2} \qquad (1)$$

The relationship between the original extract (OE), the real extract (RE), the apparent extract (AE) of beer and the produced ethanol A, % w/w ($A_{W/W}$) are presented in tables, produced by Balling (Balling, 1865), Wahl and Henius (Wahl and Henius, 1908), Holtzer (Holtzer,1904) and others. In the work of Cutaia et. al., 2009 the all data used by the authors to find a connection between the operational parameters of pilot plants and industrial breweries are summarized (Cutaia et. al., 2009).
The most well known expression relating OE, RE and Aw/w is the Balling equation, which relates the original extract to the real extract and alcohol (% w/w):

$$\underset{extract}{2,0665\,g} \rightarrow \underset{alcohol}{1,000\,g} + \underset{CO_2}{0,9565\,g} + \underset{biomass}{0,11\,g} \qquad (2a)$$

or:

$$OE = \frac{100*(2,0665*A_{W/W} + RE)}{\left(100 + 1,0665*A_{W/W}\right)} \qquad (2b)$$

The Balling's equation suggests, that from 2.0665 g wort extract, we received 0.11 g yeasts biomass and all sugars in the wort are fermentable monosaccharaides.
The batch fermentation rate on laboratory scale can be easily monitored via measuring the weight of fermentation bottles (flasks). The weight loss is connected with the release of CO_2 and the accumulation of alcohol which leads to reduction in beer specific gravity. The amount of released CO_2 in fermentation on semi-industrial and industrial scale can be easy measured. In both cases, if the relationship between the

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amounts of released CO_2 (weight loss of samples) and the amounts of fermented sugars and produced alcohol is known, the progress of the fermentation can be easily investigated.

The aim of the present study was to formulate a mathematical model for description of beer fermentation on the basis of the weight loss of fermentation bottles and to find a relationship between the amount of CO_2 and the brewing parameters – OE, AE, RE, $A_{W/W}$. The kinetics parameters of the process were determined on the basis of the received results using our model.

MICROORGANISMS AND FERMENTATION CONDITIONS

The fermentation was carried out with top-fermenting yeasts *Saccharomyces cerevisiae* S-33 and bottom-fermenting yeasts *Saccharomyces cerevisiae* S-23. The wort with 3 different original extracts – 9, 11 and 13% was used for fermentations. All media were sterilized at 121 °C for 20 min before fermentations.

The cells were immobilized in a 3 % 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 10^7 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 number of beads were placed into 0,38 % (w/v) chitosan solution in 1% acetic acid (v/v). Alginate beads stayed in chitosan solution for 60 min. Afterwards, chitosan-alginate beads are washed with physiological solution (saline) to remove the excess of chitosan. Then the beads was transferred in in 0,05 M Na-citrate solution for 30 min for constructing microcapsules with liquid core. Afterwards, chitosan-alginate beads with liquid core were washed with physiological solution (saline) (Willaert 2001).

The fermentation was made with free and immobilized cells. The fermentation was held in bottles, containing 400 ml sterile wort, equipped with fermentation stoppers. Every bottle was inoculated with 0.33 g dry yeasts or 14 g of immobilized beads. The fermentation was carried out in temperature controlled room at 15 °C for 240 h. The fermentation processes were monitored via the amount of released CO_2, which is measured from the weight loss at 24 h. The shown results were average from 3 parallel processes.

The ethanol and extract concentrations are determined by a specialized apparatus type „Anton Paar DMA 4500", Austria. This is a standard method from EBC-analityca (European Brewery Convention, Analytica EBC 2005). The biomass concentration was determined by measuring OD 600 by „Shimatzu UV-VIS 1800". The biomass concentration in immobilized cells was determined using the following methodology: 1.0 g of beads with immobilized cells was put into 1 M solution of magnesium citrate for dissolving of beads; for the control probe 1 g of pure beads were put into 1 M magnesium citrate; after releasing of biomass its concentration was determined spectrophotometrically (Zhou et. al. 1998).

MATHEMATICAL MODELS AND THEIR INTERPREATION

The main relationship, which is used in brewing, is Balling's equation (2a). On the base of this the following coefficients for the process were received:

$$\begin{aligned} Y_{P/S} &= 1{,}0000\ \underset{alcohol}{g} \Big/ 2{,}0665\ \underset{extract}{g} = 0{,}4839\ \text{g/g};\\ Y_{X/S} &= 0{,}11\ \underset{biomass}{g} \Big/ 2{,}0665\ \underset{extract}{g} = 0{,}0532\ \text{g/g};\\ Y_{CO_2/S} &= 0{,}9565\ \underset{CO_2}{g} \Big/ 2{,}0665\ \underset{extract}{g} = 0{,}4628\ \text{g/g} \end{aligned} \tag{2c}$$

The fermentation process was described with the following system of ordinary differential equations:

$$\begin{cases} \dfrac{dX}{dt} = \mu(t)X(t) \\ \dfrac{dP}{dt} = q(t)X(t) \\ \dfrac{dS}{dt} = -\dfrac{1}{Y_{x/s}}\dfrac{dX}{dt} - \dfrac{1}{Y_{p/s}}\dfrac{dP}{dt} \end{cases} \tag{3}$$

where X(t) was biomass' concentration, P(t) is ethanol concentration, S(t) was extract (substrate) concentration; $Y_{X/S}$ and $Y_{P/S}$ were yield coefficients; μ(t) and q(t) were specific growth and product accumulation rates.

Assuming Gay–Lussac relationships, the concentrations of substrate S(t) and ethanol E(t), together with *dS/dt* and *dE/dt*, can be deduced from the amount of carbon dioxide released, $CO_2(t)$, using:

$$\begin{aligned} S(t) &= S_{\text{int}} - aCO_2(t)\\ P(t) &= b(S_{\text{int}} - S(t)) \end{aligned} \tag{4}$$

where ***a*** and ***b*** are coefficients; S_{int}=OE; S(t)=RE(t).

The same approach was used for the control of fermentation process in winemaking (Goelzer et. al. 2009).

The careful consideration of the Balling's equation and the shape of the experimental curves for CO_2 enables us to determine the coefficients ***a*** and ***b*** easily. The coefficient ***b*** came directly from equation (2b) and its value was ***0.4839***. The average value of **b** depended on the yeast strain and fermentation conditions and varies from 0.48 to 0.57 (Cutaia et. al. 2009). But as a first approximation, its value from Balling's equation could be used.

The value of ***a*** could be determined experimentally, if fermentation rates were known. In series of experiments, the amount of released CO_2 and the parameters OE, AE, RE, $A_{W/W}$ were measured during fermentation. It was found that the value of ***a*** varied from 0.49-0.53, i.e. it was closer to 0.4839. Therefore, this value could also be used for determination of the extract dynamics. The determined values of coefficients **a** and **b** were taken into account in equation (4):

$$\begin{aligned} S(t) &= S_{\text{int}} - 0{,}5157CO_2(t)\\ P(t) &= 0{,}5157(S_{\text{int}} - S(t)) \end{aligned} \tag{4a}$$

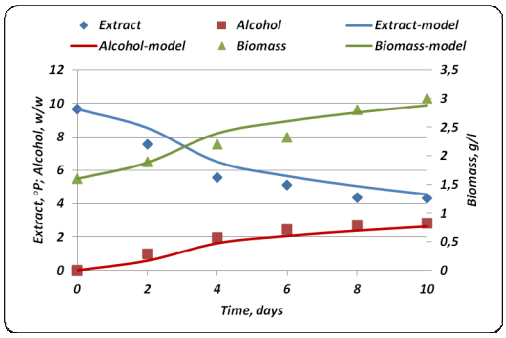
An important feature of the fermentation process is the biomass concentration in a fermentation medium. The formation of biomass is associated with cell division

process, in which CO_2 also releases. Consequently, the concentration of biomass can also be determined by the amount of released CO_2. The starting point for such calculation will be again the Balling's equation. According to it, 5.32% of the fermentable extract is used by the cells for their vital activity and formation of new cells. It was found in the series of preliminary experiments that the yield coefficient of biomass was higher and it was 10% indeed. Consequently, the concentration of biomass in the medium can be determined by the relationship:

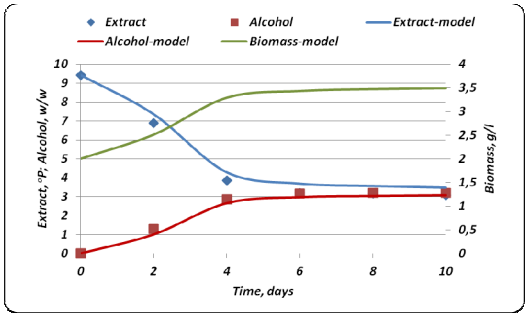
$$X(t) = 0{,}1\left[S(t)_{i-1} - S(t)_i\right] \qquad (4b)$$

where $S(t)_{i-1}$ and $S(t)_I$ are the OE of the wort;

The method was applied for determination of the fermentation parameters in beer production. The results of one of the comparisons are presented in Figure 1. Table 1 shows the statistical analysis of experimental and calculated data presented in Figure 1 (original extract 9%). The data for other investigations are similar.



a) free cells *S. cerevisiae* S-23



b) immobilized cells *S. cerevisiae* S-23

Figure 1. Comparison between model and experimental results (eq. 4, 4a, 4b); OE=9°P

Table 1

F-test for results on figure 1.

F-Test Two-Sample for Variances (Extract)		
	Experimental	***Model***
Mean	6,1033	6,656
Variance	4,424	4,158
Observations	6	6
df	5	5
F	1,064	
P(F<=f) one-tail	0,473	
F Critical one-tail	5,050	

The statistical analysis showed that the mathematical model gave correct results in calculating the basic parameters of the fermentation process. The conclusion was that the model gave accurate results for the concentration of biomass, slightly underestimated the alcohol formation and respectively, overestimated the final extract in beer. The reason was the initial values of selected parameters. Despite these disadvantages, the proposed model satisfactorily described the course of the fermentation process and it was applicable in practice.

The results for fermentation dynamics, received by equations (4a) and (4b) were used for determination of kinetic parameters of alcoholic fermentation with free and immobilized cells.

KINETICS PARAMETERS OF ALCOHOL FERMENTATION WITH FREE AND IMMOBILIZED CELLS

The fermentation process kinetics was described with the ordinary differential equation (3). The main kinetic parameters are: specific growth rate $\boldsymbol{\mu}$ and specific product accumulation rate $\boldsymbol{q}$. In present investigation three models were used - 5a, 5b, 5c (Birol et. al., 1998; Kostov et. al. 2011). The identification of parameters was made with MatLab. The software minimized the sum of squared errors of the model outputs with respect to the experimental data:

$$e = \left(X(k_1, k_2, \cdots, k_n) - X^e\right)^2 + \left(S(k_1, k_2, \cdots, k_n) - S^e\right)^2 + \left(P(k_1, k_2, \cdots, k_n) - P^e\right)^2$$

For that purpose the function "fmincon" was applied. Here k_i, $i = 1 \div n$ was vector of model parameters to be determined as output of minimization procedure. For that purpose the following complimentary differential equations:

$$dk_i/dt = 0, \quad i = 1 \div n$$

were added to the ordinary differential equations model because k_i, $i = 1 \div n$ were constants. For solving the overall differential equations system based on the explicit Runge-Kutta of 4-5 order formula using MATLAB function "ode45" (Kostov et. al. 2011; Mitev and Popova, 1995; Popova 1997). All parameters are presented on tables 2, 3 and 4.

- **Monod**

$$\mu = \mu_{\max}\frac{S}{K_{sx}+S};\quad q = q_{p\max}\frac{S}{K_{sp}+S} \qquad (5a)$$

- **Aiba**

$$\mu = \mu_{\max}\frac{S}{K_{sx}+S}\exp(-K_{ix}P)X;\qquad q = q_{p\max}\frac{S}{K_{sp}+S}\exp(-K_{ip}P)X \qquad (5b)$$

- **Tiessier**

$$\mu = \mu_{\max}\left(1-\exp\left(-\frac{S}{K_{sx}}\right)\right)\qquad q = q_{p\max}\left(1-\exp\left(-\frac{S}{K_{sp}}\right)\right) \qquad (5c)$$

Table 2

Kinetic parameters for beer fermentation with free and immobilized cells - *ORIGINAL EXTRACT – 9%*

Model parameters								*Efficiency coefficients*		*Model error*
μ_{max}	K_{sx}	q_{pmax}	K_{sp}	$Y_{x/s}$	$Y_{p/s}$	K_{ix}	K_{ip}	η_μ	η_q	
d^{-1}	$g.dm^{-3}$	$g.(g.d)^{-1}$	$g.dm^{-3}$	-	-	$g.dm^{-3}$	$g.dm^{-3}$	-	-	
Saccharomyces cerevisiae S-23 (bottom fermented yeasts)										
Monod										
Free cells										
0.424	***150***	***2.042***	***150***	***0.125***	***0.53***	-	-	***0.708***	***1.350***	***0.306***
Immobilized cells										
0.3	***57.74***	***2.756***	***166.33***	***0.063***	***0.58***	-	-			***0.123***
Tiessier										
Free cells										
0.412	***200***	***1.796***	***100***	***0.167***	***0.6***	-	-	***1.534***	***2.132***	***0.07***
Immobilized cells										
0.633	***200***	***2.292***	***150***	***0.148***	***0.58***	-	-			***0.272***
Aiba										
Free cells										
0.521	***158.672***	***4.07***	***161.419***	***0.125***	***0.56***	***0.155***	***0.06***	***1.741***	***1.954***	***0.224***
Immobilized cells										
0.907	***145.63***	***6.21***	***150.21***	***0.115***	***0.531***	***0.199***	***0.09***			***0.81***
Saccharomyces cerevisiae S-33 (top fermented yeasts)										
Monod										
Free cells										
0.157	***200***	***3.39***	***200***	***0.0597***	***0.521***	-	-	***1.261***	***0.914***	***0.416***
Immobilized cells										
0.196	***226.71***	***3.1***	***216.72***	***0.063***	***0.555***	-	-			***0.382***
Tiessier										
Free cells										
0.181	***200***	***1.754***	***100***	***0.086***	***0.444***	-	-	***0.883***	***0.886***	***0.577***
Immobilized cells										
0.159	***200***	***1.554***	***100***	***0.053***	***0.593***	-	-			***0.382***
Aiba										
Free cells										
0.165	***173.2***	***2.321***	***152.11***	***0.085***	***0.532***	***0.231***	***0.427***	***0.794***	***1.877***	***0.725***
Immobilized cells										
0.131	***232.1***	***4.357***	***183.12***	***0.088***	***0.572***	***0.133***	***0.674***			***0.621***

In the present work, it was accepted that the fermentation with immobilized cells could be modeled using the common relations for free cells. The influence of diffusion resistances in the system was described by the efficiency coefficients, which generalize the influence of internal and external diffusion resistances - η_μ (related to the maximal specific growth rate) and η_q (related to maximal specific ethanol production rate) defined as follows:

$$\eta_\mu = \frac{\mu_{\max}^{free}}{\mu_{\max}^{imm}} \quad \eta_q = \frac{q_{\max}^{imm}}{q_{\max}^{free}}$$

The results from the process kinetics led to interesting conclusions. First, the specific growth rate of the investigated yeasts strains decreased, when the original extract increased.

Second, there were interesting results, when wort with low original extracts was used. Immobilized cells of yeast strain S-23 showed higher specific growth rates than free cells. The main reason was increased concentration of cells in the working volume, which together with low original extract of wort led to higher specific rate of fermentation. The same was observed when wort with 11% OE was used. In contrast to the bottom fermenting yeast, the immobilized cells of top fermenting yeast strain S-33 showed lower specific growth rate than free cells. The difference between specific growth rates of immobilized and free cells was minor at wort with 9% OE, i.e. immobilization did not significantly effect on the cell growth and on the fermentation process. An important indicator of fermentation kinetics is the specific rate of accumulation of ethanol in the medium, which varies between 0.6 and 6 g /(g.d) ethanol. The studied yeast strains showed major differences in this parameter. The fermentation with bottom fermenting yeast ran smooth, without abrupt changes of ethanol content in the medium. The produced beers were with well-formed flavor profile. The fermentation with top fermenting strain S-33 was rapid and the ethanol was accumulated mainly at the first 2-3 days of primary fermentation. At that time larger amounts of secondary metabolites were also accumulated, which had to be reduced in the next

stage of fermentation. At the end of fermentation beers were with well-balanced flavor.

The fermentation rate depends on temperature of fermentation. The optimal fermentation temperatures are: 15-22°C for top-fermenting yeast strain and 8-18°C for bottom-fermenting strains. Therefore, differences in fermentation process were observed.

We can conclude that the fermentation with immobilized cells was faster at the end, which led to higher fermentation degree of produced beers. These beers were characterized by watery taste, because of lower non-fermented extract.

The influence of process immobilization on the investigated yeasts strains was different. The more interesting results were received for the top-fermenting strain – S-33. There was a reduction of the kinetics parameters for the immobilized cells. So, we could conclude that the immobilization had a significant effect on the fermentation process.

Table 3

Kinetic parameters for beer fermentation with free and immobilized cells - *ORIGINAL EXTRACT – 11%*

Model parameters								*Efficiency coefficients*		*Model error*
μ_{max}	K_{sx}	q_{pmax}	K_{sp}	$Y_{x/s}$	$Y_{p/s}$	K_{ix}	K_{ip}	η_u	η_q	
d^{-1}	$g.dm^{-3}$	$g.(g.d)^{-1}$	$g.dm^{-3}$	-	-	$g.dm^{-3}$	$g.dm^{-3}$	-	-	
Saccharomyces cerevisiae S-23 (bottom fermented yeasts)										
Monod										
Free cells										
0.117	*150*	*2.03*	*150*	*0.093*	*0.53*	-	-	*2.564*	*2.724*	*0.725*
Immobilized cells										
0.30	*204.26*	*5.53*	*226.71*	*0.6*	*0.58*	-	-			*0.209*
Tiessier										
Free cells										
0.114	*200*	*0.653*	*0.344*	*0.039*	*0.6*	-	-	*2.228*	*3.987*	*0.747*
Immobilized cells										
0.254	*200*	*2.604*	*100*	*0.06*	*0.6*	-	-			*0.371*
Aiba										
Free cells										
0.348	*173.12*	*3.037*	*143.17*	*0.13*	*0.55*	*0.055*	*0.213*	*2.201*	*2.073*	*0.203*
Immobilized cells										
0.766	*200*	*6.296*	*200*	*0.13*	*0.56*	*0.070*	*0.027*			*0.587*
Saccharomyces cerevisiae S-33 (top fermented yeasts)										
Monod										
Free cells										
0.294	*200*	*6.37*	*197.11*	*0.054*	*0.581*	-	-	*0.918*	*0.695*	*0.202*
Immobilized cells										
0.270	*226.71*	*4.43*	*226.72*	*0063*	*0.57*	-	-			*0.18*
Tiessier										
Free cells										
0.304	*210.13*	*3.010*	*100*	*0.057*	*0.060*	-	-	*0.651*	*0.729*	*0.321*
Immobilized cells										
0.198	*205.7*	*2.195*	*92.11*	*0.0623*	*0.6*	-	-			*0.116*
Aiba										
Free cells										
0.450	*62.68*	*5.907*	*100*	*0.063*	*0.65*	*0.086*	*0.035*	*1.564*	*0.789*	*0.131*
Immobilized cells										
0.704	*200*	*4.662*	*200*	*0.125*	*0.56*	*0.066*	*0.021*			*0.733*

The change in yield coefficients was important for practical application of immobilized cells. The biomass yield coefficient varied between 0.05 and 0.17, which confirmed the initial conclusions – more than 5% of the OE converted to biomass. It can be summarized that the average biomass yield coefficient is 10-11%, i.e. 0.1 to 0.11. The degree of fermentation of all experimental data varied between 55 and 65%. The observed differences were due to the quantity of fermentable extract in the medium and the fermentation rate.

It had to be highlighted that there was no strong substrate or product inhibition. The coefficients in Aiba's model were close to zero and the model was close to the general equation of Monod. It was interesting that Aiba's model gave slightly higher values than the Monod's model. The reason was an additional article, taking into account the substrate or product inhibition. Although this element was negligible, it should not be excluded.

The accuracy of models for description of experimental data was similar. The studies on the accuracy of the models showed that the pattern of Monod and Tissier gave satisfactory accuracy for this stage of work. From the obtained results it is difficult to choose the only one

model. The three mathematical relationships are characterized by their simplicity and good approximating capability. In terms of proper synthesis models of such systems should be characterized by its simplicity as offer the four models. These relationships described very well the fermentation process and gave a clear idea of the process parameters' influence on the kinetic characteristics. In practice the Aiba model could be simplified to the Monod equation because of weak product inhibition.

CONCLUSION

The work presents a method for determining the basic parameters of the alcoholic fermentation process in brewing, based on the amount of produced CO_2. The method is based on the known Balling's equation and the experiment data for the fermentation processes. The developed method is applicable in laboratory and industrial practice and gives reliable results with a good description of the fermentation process. The kinetics of the fermentation process was determined using three mathematical models. There are differences in the course of fermentation with free and immobilized top and bottom fermenting yeast strains. The influence of immobilization on fermentation with two different strains leads to a different taste and flavor profile of the produced beers.

Table 4

Kinetic parameters for beer fermentation with free and immobilized cells - *ORIGINAL EXTRACT – 13%*

Model parameters								***Efficiency coefficients***		***Model error***
μ_{max}	K_{sx}	q_{pmax}	K_{sp}	$Y_{x/s}$	$Y_{p/s}$	K_{ix}	K_{ip}	η_u	η_q	
d^{-1}	$g.dm^{-3}$	$g.(g.d)^{-1}$	$g.dm^{-3}$	-	-	$g.dm^{-3}$	$g.dm^{-3}$	-	-	
Saccharomyces cerevisiae S-23 (bottom fermented yeasts)										
Monod										
Free cells								***1.339***	***1.259***	
0.224	***150***	***3.839***	***150***	***0.124***	***0.476***	-	-			***0.133***
Immobilized cells										
0.300	***221.420***	***4.901***	***226.72***	***0.063***	***0.58***	-	-			***0.370***
Tiessier										
Free cells								***1.092***	***1.02***	
0.27	***200***	***2.437***	***100***	***0.0621***	***0.6***	-	-			***0.691***
Immobilized cells										
0.294	***175***	***2.485***	***12.3***	***0.0527***	***0.7***	-	-			
Aiba										
Free cells								***0.620***	***0.823***	
0.527	***200***	***6.665***	***200***	***0.125***	***0.512***	***0.0361***	***0.023***			***0.143***
Immobilized cells										
0.327	***200***	***5.487***	***200***	***0.081***	***0.56***	***0.009***	***0.009***			***0.102***
Saccharomyces cerevisiae S-33 (top fermented yeasts)										
Monod										
Free cells								***0.559***	***0.879***	
0.395	***221.214***	***3.297***	***200***	***0.065***	***0.61***	-	-			***0.192***
Immobilized cells										
0.221	***158.11***	***2.897***	***200***	***0.071***	***0.59***	-	-			***0.210***
Tiessier										
Free cells								***0.597***	***0.708***	
0.307	***178.12***	***2.995***	***150***	***0.074***	***0.597***	-	-			***0.347***
Immobilized cells										
0.221	***199.78***	***2.123***	***150***	***0.075***	***0.61***	-	-			***0.214***
Aiba										
Free cells								***2.038***	***0.747***	
0.321	***124.21***	***6.130***	***100***	***0.054***	***0.59***	***0.123***	***0.065***			***0.464***
Immobilized cells										
0.654	***200***	***4.578***	***164***	***0.101***	***0.55***	***0.094***	***0.009***			***0.214***

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