ANALYTICAL APPROACHES FOR DETERMINING THE EFFECTS OF WORT EXTRACT ON THE SPECIFIC GROWTH RATE OF THE YEAST POPULATION

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KEYWORDS

brewing, kinetics of biomass growth, high gravity fermentation

ABSTRACT

The effect of the original wort extract on the biomass growth rate was examined in the present work. For this purpose, analytical dependencies to determine the kinetic parameters that reveal different sides of the fermentation process, have been used. The joint influence of the wort extract and the immobilization process on the growth of the microbial population as well as on the accuracy of the analytical dependences used to describe the microbial growth kinetics in the yeast population was established by analyzing the kinetics of the fermentation process.

INTRODUCTION

Basic processes in beer production

The main stages of the brewing process are wort production (mashing, filtering, boiling, clarification and cooling), fermentation (consisting of main fermentation and beer maturation processes), and processes related to the processing and stabilization of the finished beer (Kunze 2003). The main stage in beer production is the process of alcohol fermentation. Ethanol fermentation occurs as a result of the yeast enzymatic activity in the 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).

Fermentation and maturation are the longest processes in brewing. The main fermentation lasts between 3-6 days and the maturation lasts up to 2 weeks depending on the fermentation type and the equipment used. On such a competitive market, the potential time savings offered by immobilized cell technology (ICT) have to be taken into account. Immobilized cells are physically limited or localized in a specific space while preserving their catalytic activity, and if possible, and even necessary, viability, and which can be used repeatedly and continuously (Godia et al., 1987; Shopska et al., 2019). The use of immobilized cell systems offers a number of advantages (Boulton and Quain, 2001; Hayes et al., 1991; Shopska et al., 2019): increased cell concentration in the reactor operating volume and increased reaction rate; smaller bioreactor sizes, and in continuous processes - lower reaction times; easy separation and regeneration of biomass; possibility for use in both batch and continuous alcohol fermentation. Immobilization is characterized by the following disadvantages (Mensour et al., 1996; Shopska et al., 2019): limited mass exchange due to the presence of diffusion resistances; possibility of destroying the matrix at a high rate of the fermentation process and/or the formation of gaseous metabolites; changes in the physiological state of the immobilized culture as well as in the growth rate and the overall stoichiometry of the reactions in the immobilized system. Immobilized yeast cell technology allows beer production to be accomplished in as little as 2-3 days (Branyik et al, 2005). Immobilized cell systems are heterogeneous systems in which considerable mass transfer limitations can occur, resulting in a changed yeast metabolism (Willaert, 2007). Consequently, the main challenge for ICT is to reproduce the traditional beer flavor.

The fermentation rate depends largely on the original wort extract. The increase in the wort extract leads not only to prolongation of the fermentation time, but also to changes in both primary and secondary yeast metabolism. In recent years, the so-called. high gravity brewing was introduced. The wort has a high original extract (15-17.5 °P). The final value of the extract depends on biochemical and microbiological limitations. The increase in the original extract has a negative effect on the fermentation process and the quality of the finished beer (Kunze 2003).

Equation of fermentation

The process of alcohol fermentation in beer production can be described with the following system of differential equations (eq. 1). The Monod equation (eq. 2 and eq.3) can be used to describe the kinetics of microbial growth in beer production. The parameter determination in the equation can be done analytically or by solving the differential growth equation by numerical methods. In our previous work (Kostov et al., 2019), we described analytical approaches to determine the yeast specific growth rate. Out of the methods discussed in our previous work, the methods of Warpholomeew-Gurevich Linearization and Linearization by Substrate Consumption and Product Accumulation were most suitable for describing the fermentation process in the brewing industry (Kostov et al., 2019).

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$$\begin{cases} \frac{dX}{d\tau} = \mu X \\ \frac{dP}{d\tau} = qX \\ \frac{dS}{d\tau} = -\frac{1}{Y_{X/S}}\frac{dX}{\tau} - \frac{1}{Y_{P/S}}\frac{dP}{d\tau} \\ \frac{dE}{d\tau} = Y_E \mu X \\ \frac{dHA}{d\tau} = Y_{HA}\mu X \\ \frac{dA}{d\tau} = Y_A \mu X - k_A XA \\ \frac{dVDK}{d\tau} = Y_{VDK}\mu X - k_{VDK} XVDK \end{cases} \quad (1)$$

where: X – biomass concentration, g/dm^3; P – ethanol concentration, g/dm^3; S – real extract, g/dm^3; $Y_{P/S}$, $Y_{X/S}$ – yield coefficients; μ – specific growth rate, h^{-1}; q – specific ethanol accumulation rate, g/(g.h); E – ester concentration, mg/dm^3; HA – higher alcohol concentration, mg/dm^3; A – aldehyde concentration, mg/dm^3; VDK – vicinal diketone concentration, mg/dm^3; Y_{HA}, Y_E, Y_A, Y_{VDK} – yield coefficients of the corresponding metabolites, mg/(g.h); k_A, k_{VDK} – reduction coefficients for aldehydes and vicinal diketones, mg/(g.h); K_{SX}, K_{SP} – Monod constants, g/dm^3; K_{SXi} , K_{SPi} – inhibition constants, g/dm^3; P_M, P_{MP} – maximal ethanol concentration for full inhibition of the process, g/dm^3

$$\frac{dX}{d\tau} = \mu X \quad (2)$$

$$\mu = \mu_{max}\frac{S}{K_S + S} \quad (3)$$

where: μ_{max} - maximum specific biomass growth rate, h^{-1}; K_S - saturation constant, g/dm^3.

Warpholomeew-Gurevich Linearization

For the determination of the kinetic parameters in equation (3), the approach suggested by Warpholomeew-Gurevich can also be used. In this case, the experimental parameters are represented by modifications of equations (2) and (3) as follows (Warpholomeew and Gurevich. 1999):

$$\frac{\ln\left(\frac{X}{X_0}\right)}{\tau} = \frac{\mu_{max}S_0}{K_S + S_0} + \frac{K_S}{K_S + S_0}\frac{\ln\left(\frac{X_F - X}{X_F - X_0}\right)}{\tau} \quad (4)$$

where: X_F - final biomass concentration, g/dm^3; X - current biomass concentration, g/dm^3; X_0 - initial biomass concentration, g/dm^3; S_0 and S - initial and current substrate concentration, g/dm^3.

To determine the kinetic parameters in the Monod equation, the dependence $\frac{\ln\left(\frac{X}{X_0}\right)}{\tau} = f\left(\frac{\ln\left(\frac{X_F - X}{X_F - X_0}\right)}{\tau}\right)$ is used, and the kinetic parameters in the Monod model are determined from the straight line equation.

Linearization by Substrate Consumption and Product Accumulation

Warpholomeew-Gurevich linearization approach also allows the growth rate to be determined using the changes in the substrate and the product concentration. For this purpose, equation (4) would acquire the form (Warpholomeew and Gurevich. 1999):

$$\frac{\ln\left(\frac{S_0 - S_j}{S_0 - S_i}\right)}{\tau_j - \tau_i} = \frac{\mu_{max}S_0}{K_S + S_0} + \frac{K_S}{K_S + S_0}\frac{\ln\left(\frac{S_j}{S_i}\right)}{\tau_j - \tau_i} \quad (5a)$$

$$\frac{\ln\left(\frac{P_j}{P_i}\right)}{\tau_j - \tau_i} = \frac{\mu_{max}S_0}{K_S + S_0} + \frac{K_S}{K_S + S_0}\frac{\ln\left(\frac{P_m - P_j}{P_m - P_i}\right)}{\tau_j - \tau_i} \quad (5b)$$

where S_0; S_j; S_i - initial substrate concentration, g/dm^3; substrate concentration corresponding to t_j, g/dm^3; substrate concentration corresponding to t_i, g/dm^3, τ_j-t_i - time interval equal to the difference between the final and the current process time, h; P_j; P_i – product concentration corresponding to t_j, g/dm^3; product concentration corresponding to t_i, g/dm^3, P_m - maximum concentration of the product, g/dm^3.

The equations are plotted in the corresponding coordinates $\frac{\ln\left(\frac{S_0 - S_j}{S_0 - S_i}\right)}{\tau_j - \tau_i}$ of $\frac{\ln\left(\frac{S_j}{S_i}\right)}{\tau_j - \tau_i}$; and $\frac{\ln\left(\frac{P_j}{P_i}\right)}{\tau_j - \tau_i}$ of $\frac{\ln\left(\frac{P_m - P_j}{P_m - P_i}\right)}{\tau_j - \tau_i}$.

The parameter K_S is determined from the angular coefficient of the straight lines, while μ_{max} is determined from the cut-off.

The purpose of the present work was to investigate the influence of the original wort extract on the accuracy of the analytical models for describing the fermentation process kinetics. The aim was to define an analytical approach making it is easy, accurate and quick to look for the influence of a parameter on the fermentation process kinetics and to make comparisons between different fermentation regimes. In addition, the effect of the immobilization process on the yeast primary metabolism kinetics was investigated.

MICROORGANISMS AND FERMENTATION CONDITIONS

The fermentations were carried out with top-fermenting yeast strain *Saccharomyces pastorianus* (*carlsbergensis*) S-23. Wort with 5 different original extracts – 9, 11, 13, 15 and 17 % was used for fermentations. All media were sterilized at 121 °C for 20 min before fermentations. The immobilization procedure and the fermentation conditions were previously reported in (Parcunev et.al. 2012). In this study the fermentations with free and immobilized cells were investigated for the same time intervals to determine the impact of immobilization on the yeast metabolism. Biomass concentration of immobilized cells was determined according Parcunev et.al. 2012.

The fermentation of all variants was carried out in plastic bottles, with a volume of 500 cm^3, equipped with an airlock system. The 400 cm^3 of wort was placed into the bottles and inoculated with yeast suspension at a concentration of 10^7 cfu/cm^3. For the variants with immobilized cells, the microcapsule mass was 15 g for 400 cm^3 wort.

All fermentation processes were carried out at a constant temperature (main fermentation and maturation) of 15 °C in order to avoid the effect of temperature on biochemical reactions during fermentation.

The characterization of wort, green beer and beer (OE, degree of attenuation, extract, and alcohol) was

conducted according to the current methods recommended by the European Brewery Convention (Analytica-EBC, 2004).

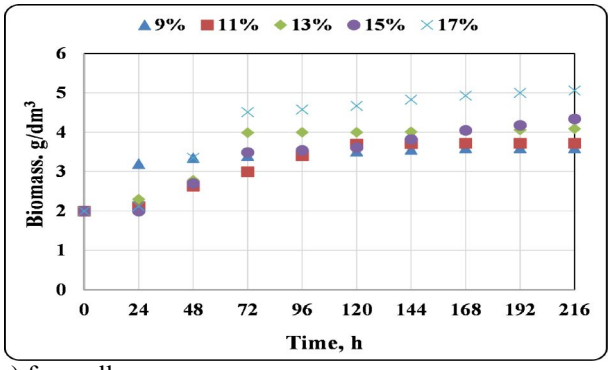
The efficiency coefficient was used to determine the impact of the immobilization process on the yeast biomass growth:

$$\eta = \frac{\mu_{IM}}{\mu_F} \quad (6)$$

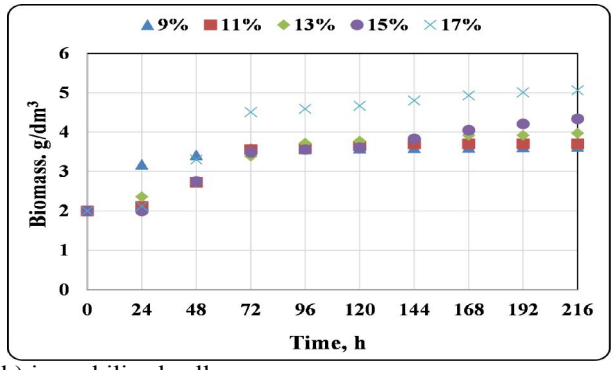
where: η- efficiency coefficient; μ_{IM} - maximum specific biomass growth rate for immobilized cells, h^{-1}; μ_F - maximum specific biomass growth rate for free cells, h^{-1};

RESULTS AND DISCUSSION

Before considering the possibilities for modeling the biomass specific growth rate at different initial values of the wort extract, it is necessary to consider the dynamics of biomass formation. The results for biomass accumulation in free cell fermentation and immobilized cell fermentation are presented in Fig. 1.



a) free cells



b) immobilized cells

Figure 1: Biomass concentration dynamics

The increase in the extract also provoked longer biomass growth. At low original extracts, the cells entered the stationary phase rather quickly, while prolongation of biomass growth in the high gravity wort was observed. The immobilization process did not generally change the observed trends, but there was a slight extension of the lag phase. Due to the increased cell volume in the fermentation volume, the main fermentation ended faster, i.e. a decrease in the total fermentation time was observed. Another important observation that is relevant to the accuracy of the models used is the fact that smaller biomass amounts accumulated in the capsules than in free cell fermentation for the same fermentation time. This was due to the physical limitations of the cell growth inside the capsules. This, in turn, would affect both the specific growth rate and the accuracy of the models.

Warpholomeew-Gurevich Linearization

The results for the linearization by the Warpholomeew-Gurevich method are presented in Fig. 2 and Table 1. Fig. 2 shows the linear dependencies on the basis of which the kinetic parameters are determined (Table 2). The data show that the original extract directly affected the kinetic parameters determined according to the proposed method. The data in Table 1 confirm the fact

Data on free cell accumulation indicate that the biomass concentration increased with the increase in the original wort extract. This is logical since the wort with higher original extract contains more fermentable carbohydrates. On the other hand, the process lag phase increased with the increase in the original wort extract, and although a relatively close biomass concentration was found at the end of the fermentation process, it is likely that the increase in the lag phase would be reflected in the accuracy of the the models. The results show that the cells adapted relatively quickly to the fermentation conditions at low original extracts - 9% and 11%. Under these conditions, the lag phase was about 24 hours, after which the cells began active fermentation. In this case, the main fermentation ended in about 3-4 days. With the increase in the extract, the duration of the lag phase increased up to 48 hours, and it was the longest in the high gravity wort - 15% and 17%. This in turn provoked an extension of the fermentation process and the main fermentation took 4-6 days.

that the kinetic parameters could be determined according to the proposed method, since the value of the saturation constant K_S is in the same order as the wort extract. This, in turn, confirms the observations of Warpholomeew-Gurevich (Warpholomeew and Gurevich. 1999) that constants in the Monod equation can only be determined if the free member in the linear equation is less than 1, i.e. the saturation constant is in the order of the substrate concentration. An interesting fact is that there was obviously an optimum zone of saturation constant values where the cells grew at the highest specific growth rate. This zone is in the range of 11-13% original wort extract. This was probably due to the presence of more glucose in the wort. The accuracy of the model is greatly improved with the decrease in the saturation constant, i.e. the cells generally grow at a higher specific growth rate.

This trend was maintained in immobilized cell fermentation, but there was a shift in accuracy to wort with 13% and 15% wort extract. This could be explained by the presence of diffusion resistances and the need for higher substrate concentrations to overcome the diffusion barrier. In this case, the presence of greater amount of fermentable extract in the wort caused the higher concentration difference.

In general, the process of immobilization had no major adverse effect on the microbial population. With the exception of the variant at 9% original wort extract, in the other variants the efficiency coefficient was below 1, i.e. the immobilized cells were affected by the immobilization process. The expected tendency to increase the effect of immobilization on the yeast population was not confirmed. In general, however, the average biomass specific growth rate was affected negatively and decreased with the increase in the extract. This was associated with an increase in the saturation constant K_S, especially in the high gravity wort. K_S was in the range of 4-5% in the high gravity wort, which was very close to the non-fermentable extract of the wort. The saturation constant was lower and the cells grew at a higher average specific growth rate at low original extracts of up to 11-13%. These observations suggest that main fermentation might occur in a shorter time, while the high extract quite expectedly led to an increase in the main fermentation time.

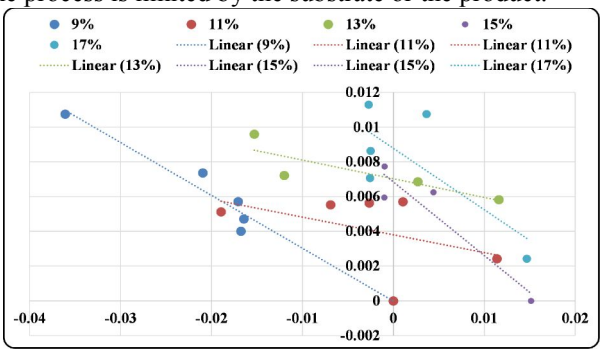
Linearization by Substrate Consumption and Product Accumulation

Linearization by substrate and product is a modification of the Warpholomeew-Gurevich Linearization, which reflect the effect of substrate consumption and product accumulation during fermentation. It reveals whether the process is limited by the substrate or the product.

Table 1: Kinetic characteristics of biomass growth determined by Warpholomeew-Gurevich Linearization

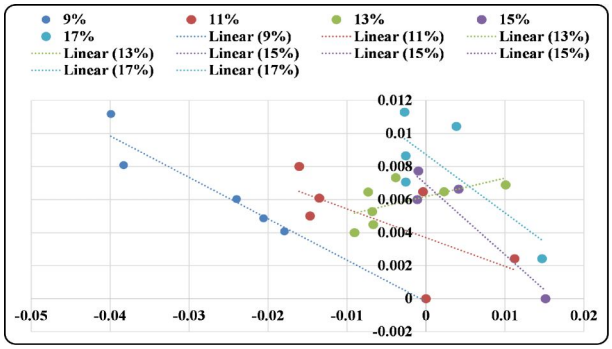
μ_{max}, h^{-1}	K_S, g/dm^3	μ_{max}, h^{-1}	K_S, g/dm^3
Free cells		**Immobilized cells**	
9%			
0.000029	39.35	0.00025	23.31
R^2=0.9669		R^2=0.9231	
η=8.621			
11%			
0.0047	30.66	0.0042	12.51
R^2=0.9521		R^2=0.8932	
η=0.894			
13%			
0.0078	15.58	0.0068	12.98
R^2=0.9123		R^2=0.9523	
η=0.872			
15%			
0.0127	41.92	0.0089	44.81
R^2=0.9359		R^2=0.9612	
η=0.700			
17%			
0.0114	31.87	0.0109	44.30
R^2=0.8232		R^2=0.9326	
η=0.956			

* correlation coefficients between the experimental biomass concentration and the calculated biomass concentration were calculated using the kinetic parameters obtained.



y_9=-0.3042x-2E-05; R^2 = 0.9595
y_{11}=-0.2181x+0.0037; R^2 = 0.4179
y_{13}=-0.1077x+0.007; R^2 = 0.7284
y_{15}=0.3879x+0.0099; R^2 = 0.964
y_{17}=-0.1579x+0.0096; R^2 = 0.2873

a) free cells



y_9=-0.2507x-0.0002; R^2 = 0.9476
y_{11}=-0.1021x+0.0038; R^2 = 0.1894
y_{13}=0.1111x+0.0062; R^2 = 0.3630
y_{15} =-0.4259x+0.0069;R^2 = 0.8762
y_{17}=0.3525x+0.0087; R^2 = 0.5771

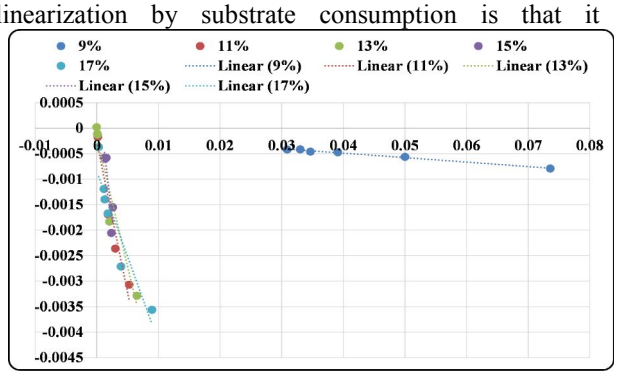
b) immobilized cells

on graph – by axis X - $\frac{\ln\left(\frac{X_F - X}{X_F - X_0}\right)}{\tau}$; by axis Y - $\frac{\ln\left(\frac{X}{X_0}\right)}{\tau}$

Figure 2: Warpholomeew-Gurevich Linearization

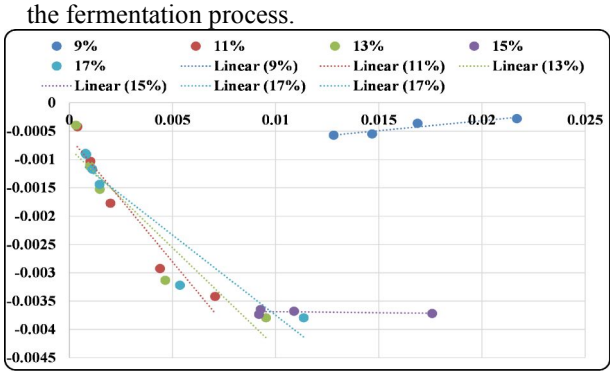
The results of the two linearization methods are presented in Fig. 3 and Fig. 4, and the kinetic parameters obtained are presented in Table 2 and Table 3. In free cells, the linearization accuracy was extremely high. This indicated that the fermentation process was substrate dependent. The data in Table 2 show a decrease in the average specific growth rate with an increase in the wort extract. Once again, two zones can be highlighted - up to 13-15% wort extract and over 15% wort extract. Cells grew at a high average growth rate in the first zone, while the average rate decreased in the second zone. This was due to an increase in the saturation constant, which was associated with an increase in the wort extract. The effect of the substrate consumption should be explained by the process of catabolic repression in yeast. In this process, cells can consume complex carbohydrates - maltose and maltotriose only after glucose is consumed (its concentration in the wort must decrease below 2%). Therefore, the substrate uptake model can be considered as a function reflecting this process.

The model using substrate consumption more clearly emphasizes the importance of immobilization. In this case, all efficiency ratios were higher than 1, reflecting the fact that the fermentation process was completed faster in the immobilized cell fermentation. The increased amount of cells in the fermentation volume decreased the importance of the catabolite repression, i.e. the cells grew at a higher specific growth rate (Table 2). Another important feature of the model by the linearization by substrate consumption is that it incorporates all experimental data from the end of the lag phase to the stationary phase, which increases its accuracy. The model accuracy is only affected in the zone of transition to fermentation of high gravity wort with an original extract of 15%. This is due to the fact that at that value the process begins to slow down due to the influence of substrate inhibition and the delayed maltose absorption in this case. This peculiarity should be taken into account when using the model to describe the fermentation process.



y_9=-0.0088x-0.0001; R^2 = 0.9913
y_{11}=-0.5911x-0.0003; R^2 = 0.9434
y_{15}=-0.5069x-0.0002; R^2 = 0.9447
y_{15}=-1.0262x+0.0008; R^2 = 0.8223
y_{17}=-0.3347x-0.0008; R^2 = 0.8786

a) free cells

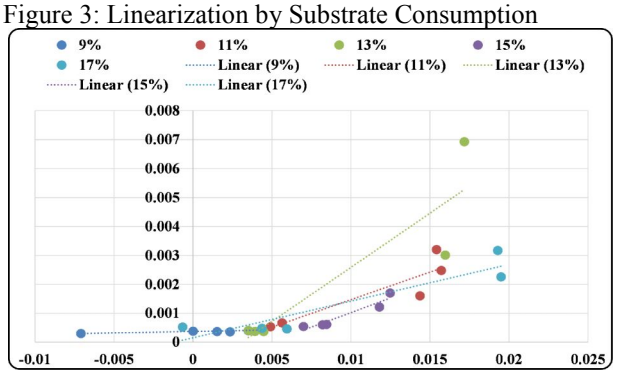


y_9=0.0352x-0.001; R^2 = 0.8922
y_{11}=-0.4401x- 0.0006; R^2 = 0.9292
y_{13}=-0.351x-0.0008; R^2 = 0.8859
y_{15} =-0.0035x-0.0037;R^2 = 0.1214
y_{17}=-0.2835x+0.0009; R^2 = 0.8930

b) immobilized cells

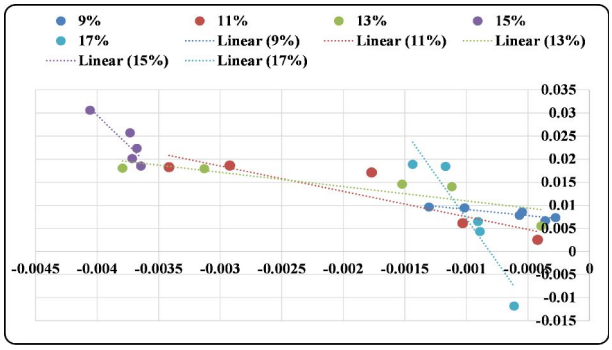
on graph – by axis X - $\frac{\ln\left(\frac{S_j}{S_i}\right)}{\tau_j - \tau_i}$; by axis У - $\frac{\ln\left(\frac{S_0 - S_j}{S_0 - S_i}\right)}{\tau_j - \tau_i}$

Figure 3: Linearization by Substrate Consumption



y_9=0.0101x+0.0004; R^2 = 0.742
y_{11}=0.1911x-0.0004; R^2 = 0.8139
y_{13}=0.3744x-0.0012; R^2 = 0.8156
y_{15}=0.2020x-0.001; R^2 = 0.9138
y_{17}=-0.1274x+0.0001; R^2 = 0.8555

a) free cells



y_9=-2.691x+0.0064; R^2 = 0.8303
y_{11}=-5.5013x+0.002; R^2 = 0.8223
y_{13}=-3.0981x+0.0079; R^2 = 0.7435
y_{15} =-25.81x+0.0737;R^2 = 0.8003
y_{17}=-37.802x+0.0306; R^2 = 0.8802

b) immobilized cells

on graph – by axis X - $\frac{\ln\left(\frac{P_m - P_j}{P_m - P_i}\right)}{\tau_j - \tau_i}$; by axis У - $\frac{\ln\left(\frac{P_j}{P_i}\right)}{\tau_j - \tau_i}$

Figure 4: Linearization by Product Accumulation

The kinetic parameters determined by the linearization by product accumulation largely confirm the previously described trends. This indicates that ethanol accumulation in the medium, which is associated with primary yeast metabolism, is a paramount process during fermentation. It is interesting that in free cell fermentation the specific growth rates determined were one order of magnitude higher than those determined by linearization by substrate consumption.

This proves again that the beer production process is substrate dependent and strongly depends on the order of consumption of carbohydrates. The accuracy of the linearization by product accumulation also depends on the inclusion of data mainly on the main fermentation process. This means that prolonged fermentation

processes using high-gravity wort led to the reduction of the model accuracy. One of the conclusions drawn from these observations and from previous studies (Kostov et al., 2019) is the following: if modeling the effect of the wort extract, it is good to use linearization by substrate consumption, but if comparing fermentation conditions (influence of temperature, pH, oxygen amount, etc.), it is good to apply linearization by product accumulation, but only on condition that the wort has the same qualitative and quantitative composition.

Table 2: Kinetic characteristics of biomass growth determined by Substrate consumption linearization

μ_{max}, h^{-1}	K_S, g/dm^3	μ_{max}, h^{-1}	K_S, g/dm^3
Free cells		**Immobilized cells**	
9%			
0.0001	0.96	0.00100	0.31
R^2=0.9921		R^2=0.9231	
η=9.947			
11%			
0.00041	40.87	0.0007	33.62
R^2=0.9236		R^2=0.8962	
η=1.904			
13%			
0.000267	43.73	0.0010	33.77
R^2=0.8932		R^2=0.8859	
η=3.771			
15%			
0.00121	75.95	0.0037	0.52
R^2=0.8631		R^2=0.214	
η=3.080			
17%			
0.00100	42.63	0.00101	37.55
R^2=0.8232		R^2=0.8621	
η=1.098			

* correlation coefficients between the experimental biomass concentration and the calculated biomass concentration were calculated using the kinetic parameters obtained.

Perhaps the most unexpected results are obtained by applying linearization by product accumulation to the immobilized cell fermentation process (Table 3). The specific growth rates obtained are between 7 and 527 times higher than those in the free cell fermentation. The results obtained more closely approximate the specific ethanol accumulation rate determined in Parcunev et al., 2012. Analogously, the data obtained for free cell fermentation also approximate those results. We can assume that actually the so-called specific growth rate is the specific rate of product accumulation. Furthermore, the analytical solution of the differential equation does not account for the diffusion of ethanol from the capsules to the fermentation medium. Probably the values obtained somehow include the diffusion coefficients of the product in the fermenting liquid, but this would be a subject to future studies.

The results in Table 1 to Table 3 show that some of the models were characterized by low accuracy of the description of the fermentation process. This was due to the inability of these models to evaluate the influence of some parameters, such as substrate and product diffusion to and from the yeast cells through the immobilization matrix, the effect of some of the metabolic products on the yeast cell growth, etc. Usually, the change in a model accuracy is on the verge of whether the beer wort is considered highly extractive or not. Despite these problems, the models could be used to quickly and accurately determine kinetic parameters and to make comparisons between different fermentation regimes with both free and immobilized cells.

Table 3: Kinetic characteristics of biomass growth determined by Product accumulation linearization

μ_{max}, h^{-1}	K_S, g/dm^3	μ_{max}, h^{-1}	K_S, g/dm^3
Free cells		**Immobilized cells**	
9%			
0.0004	1.01	0.011	65.62
R^2=0.9556		R^2=0.9264	
η=27.36			
11%			
0.00049	25.99	0.0037	93.08
R^2=0.9432		R^2=0.8264	
η=7.47			
13%			
0.00186	71.36	0.0139	98.28
R^2=0.8531		R^2=0.8146	
η=7.46			
15%			
0.0011	25.23	0.147	144.41
R^2=0.8142		R^2=0.8631	
η=123.85			
17%			
0.00011	24.82	0.0604	165.61
R^2=0.9126		R^2=0.9326	
η=527.14			

* correlation coefficients between the experimental biomass concentration and the calculated biomass concentration were calculated using the kinetic parameters obtained.

Another important feature was that the models gave up to 29 times difference in the specific growth rate (Table 1 to Table 3). This was due to the fact that they described different sides of the fermentation process. The first model described the overall biomass growth, and the second and third one provided the connection between substrate consumption, ethanol accumulation, and biomass growth. This also implied differences in the specific growth rate obtained as the models took into account the biochemical side of the process. Finally, it should be noted that the models allowed clear distinction to be made between the processes that take place in the wide range of original extracts (Table 1 to Table 3). The data shows that beer wort can be divided into three conventional groups - with "low" extract (9%), with "normal extract" (11% -13%) and with "high" extract (15% -17%). The observed difference between the kinetic parameters was due to the different composition of fermentable sugars in each of the three beer wort groups. As a result, the accuracy of the models differed in the area of transition from one group to another.

CONCLUSION

The effect of the original wort extract on three analytical methods for determining the specific growth

rate of yeast biomass was investigated. The data show that as a substrate dependent process, the kinetics of the fermentation process is best described by linearization by substrate consumption. In this case, the model takes into account in a certain way the catabolic repression that is characteristic of alcohol fermentation in brewing. Besides, it is found that the model using linearization by product accumulation can detect not the specific growth rate of the microbial population but the specific rate of product accumulation. Moreover, it is found that the linearization by metabolic product accumulation also takes into account to some extent the product diffusion from and to the immobilized preparations.

It was also found that the immobilization process was irrelevant to the accuracy of the models, and the data obtained on the efficiency coefficients confirm previous observations that the strain used was not significantly affected by the immobilization procedure.

Lastly, the accuracy of the models was found to be highly dependent on the original wort extract, with the change in the accuracy being related to the transition zone between the wort with normal extracts and the high gravity wort (15% -17% extract).

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