ANALYTICAL APPROACHES TO DETERMINE THE SPECIFIC BIOMASS GROWTH RATE IN BREWING

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ABSTRACT
Determining the specific growth rate in beer production is essential for the accuracy of the models describing the biomass accumulation kinetics. Some analytical methods for determining the specific biomass growth rate in beer production have been addressed in the present paper. Eight different methods of linearization of the microbial growth equation have been studied, and the various aspects, advantages and disadvantages of the methods have been discussed.

INTRODUCTION
Basic processes in beer production
The main stages of the brewing process are the production of wort (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 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 CO₂. In aerobic conditions, yeasts consume sugars, mainly for biomass accumulation and CO₂ production (Boulton and Quain, 2001).

Yeast metabolism during fermentation and maturation affects significantly the beer flavor. Ethanol, CO₂, esters and fusel alcohols make positive contributions to beer flavor. Dimethyl sulphide and hydrogen sulphide, diacetyl, and aldehydes contribute to the flavor defects of beer (Meilgaard, 1975). Therefore, the synthesis and reduction of yeast metabolites on the microbiological and bioengineering levels have to be studied for the purpose of yeast by-product optimization within certain limits. Fermentation and maturation are the longest processes in brewing. The main fermentation lasts between 3-6 days and the maturation 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 yeast cell technology allows the production of beer to be accomplished in as little as 2-3 days (Branyik et al, 2005; Willaert, 2007).

Equation of fermentation
The process of alcohol fermentation in beer production can be described with the following system of differential equations:

\[
\frac{dX}{dt} = \mu X
\]
\[
\frac{dP}{dt} = q X
\]
\[
\frac{dS}{dt} = \frac{1}{\tau_{\text{g}}} \frac{dX}{dt} - \frac{1}{\tau_{\text{g}}} \frac{dP}{dt}
\]
\[
\frac{dP}{dt} = Y_{\mu} P
\]
\[
\frac{dHA}{dt} = Y_{\alpha} \mu X - k_{\alpha} X A
\]
\[
\frac{dYDK}{dt} = Y_{\text{vdk}} \mu X - k_{\text{vdk}} VDK
\]

where: X – biomass concentration, g/dm³; P – ethanol concentration, g/dm³; S – real extract, g/dm³; Y_{\text{ps}}, Y_{\text{xs}} – yield coefficients; \mu – specific growth rate, h⁻¹; q – specific ethanol accumulation rate, g/(g.h); E – ester concentration, mg/dm³; HA – higher alcohol concentration, mg/dm³; A – aldehyde concentration, mg/dm³; VDK – vicinal diketone concentration, mg/dm³; Y_{\text{aha}}, Y_{\text{ya}}, Y_{\text{vdk}} – yield coefficients of the corresponding metabolites, mg/(g.h); k_{\alpha}, k_{\text{vdk}} – reduction coefficients for aldehydes and vicinal diketones, mg/(g.h); K_{xx}, K_{sp} – Monod constants, g/dm³; K_{x0}, K_{s0} – inhibition constants, g/dm³; P_{ma}, P_{max} – maximal ethanol concentration for full inhibition of the process, g/dm³

As the system of differential equations clearly shows, the accumulation of primary and secondary metabolites is associated with biomass accumulation, which makes the determination of the specific biomass growth rate critical to the accuracy of the parameter identification. The flavor-aroma profile of beer is formed by a number of compounds whose synthesis and/or reduction is directly related to the biomass state. Models of different accuracy which are subjected to various biological processes in the yeast cell have been developed for this purpose. Details of the accuracy of the models can be found in Shopska et al., 2019.

The aim of the present work was to investigate the accuracy of different types of analytical methods for determining the specific biomass growth rate during alcohol fermentation for beer production.
MICROORGANISMS AND FERMENTATION CONDITIONS

Yeast strains
Beer fermentation was carried out using a bottom-fermenting dry yeast *Saccharomyces pastorianus* (carlsbergensis) Slaflager S-23 (Fermentis, France).

Wort
Wort with original extract (OE) 13±0.5 °P was produced in a 20 L laboratory scale brewery (Braumeister, Germany). 4 kg malt and 0.5 kg barley were mixed with water at a 1:4 ratio. Mashing was conducted by increasing the temperature by 1 °C/min and by maintaining the following temperature rest: 20 min at 45 °C, 30 min at 52 °C, 40 min at 63 °C, 25 min at 72 °C and 1 min at 78 °C. Lautering and boiling were also conducted in the same Braumeister. Boiling duration was approximately 1 h, and hop granules (Nugget variety) were added to the wort at the beginning of the process.

Wort fermentation
The fermentation of all variants was carried out in plastic bottles, with a volume of 500 cm³, equipped with an airlock system. The 400 cm³ of wort was placed into bottles and inoculated with a yeast suspension at a concentration of 10⁷ cfu/cm³. For the variants with immobilized cells, the microcapsule mass was 15 g for 400 cm³ wort. The main fermentation and maturation temperature was a constant: 15 °C.

Immobilization
The immobilization and fermentation conditions were previously reported in (Parcunev et. al., 2012).

Analytical methods and procedures
The biomass concentration of the immobilized cells was determined according to the mathematical model proposed in (Parcunev et.al. 2012). The characterization of wort, green beer and beer (OE, degree of attenuation, extract, alcohol and VDK) was conducted according to the current methods recommended by the European Brewery Convention (Analytica-EBC, 2004). The metabolite concentrations were determined according to (Marinov, 2010).

LINEARIZATION METHODS FOR THE MONOD MODEL

The basic dependence that is valid for each biological process involving microorganisms is used to determine the specific growth rate ( Shopska et. al., 2019):

\[
\frac{dX}{d\tau} = \mu X
\]

Then the specific growth rate is presented in the following way:

\[
\mu = \frac{dX}{d\tau} \frac{d\tau}{X}
\]

Equation (3) can be presented in the form of a real dependence for a certain interval of time Δτ and ΔX:

\[
\mu = \frac{\frac{AX}{\Delta \tau}}{X}
\]

Equations (3) and (4) are the two equations by which the specific growth rate can be calculated, using several successive steps and different linearization methods. To determine the growth rate, it is necessary to remove the lag phase from the experimental data. In this case, μ for each local point of the study was calculated (Cibranska and Mutačiev, 2009; Fazlollah et al., 2017):

\[
\frac{(X_F - X_{fa,t})}{F_{fa,t}} = \frac{\tau - (\tau + \Delta \tau)}{X_{fa,t}}
\]

where: X_F - final biomass concentration, g/dm³; X_{fa,t} - current biomass concentration, g/dm³; τ_f - final fermentation time, h; τ + Δτ - current fermentation time, h; τ_0 - 0 h (start of the fermentation); Δτ – time change, h; - (for the correctness of the calculations Δτ=const); X_{fa,t} – biomass concentration at the beginning of the exponential phase, g/dm³;

Equation (5) suggests that the specific growth rate would be a decreasing dimension and during the steady state μ→0. This does not mean that the cells stop growing, but that the number of newly formed cells would be equal to the number of dying cells, and therefore X→const.

To determine the biomass growth kinetics the Monod equation is used:

\[
\mu = \frac{\mu_{max} S}{K_S + S}
\]

where: \( \mu_{max} \) - maximum specific biomass growth rate, h⁻¹; \( K_s \) - saturation constant, g/dm³. The kinetic parameters \( \mu_{max} \) and \( K_s \) in the Monod model can be determined by various methods of linearization of the function (2).

All linearization methods have been described by Warpholomeew and Gurevich, 1999; Cibranska and Mutačiev, 2009; Fazlollah et al., 2017; Kemmer and Keller, 2010.

Lineweeaver-Burk Linearization
This is a classical method for determining the kinetic parameters of the enzyme reaction in the Michaelis-Menten equation. The method is easy for application because the Monod equation and the Michaelis-Menten equation have the same form. For this purpose, equation (6) is transformed to:

\[
\frac{1}{\mu} = \frac{1}{\mu_{max}} + \frac{K_S}{\mu_{max} S}
\]

and the dependence 1/μ = f (1/S) is plotted. The resultant function is approximated with a linear response of the type y=ax+b, where the values of the coefficients in the straight line equation are as follows: a=KS/\mu_{max} and b=1/\mu_{max}, from which the desired kinetic parameters are determined.

Hanes Linearization
In this case, dependence (6) takes the following form:

\[
\frac{S}{\mu} = \frac{S}{\mu_{max}} + \frac{1}{\mu_{max} S}
\]

The resultant function is approximated with a linearity of the type y=ax+b, where the values of the coefficients
in the straight line equation are as follows: \(a=1/\mu_{\text{max}}\) and \(b=K_s/\mu_{\text{max}}\), from which the desired kinetic parameters are determined.

### Eadie-Hofstee Linearization

In this case, equation (6) takes the form of:

\[
\mu = \mu_{\text{max}} - \frac{K_s \mu}{S} \tag{9}
\]

Then, the dependence \(\mu=\psi(\mu/S)\) is plotted and an approximate to the straight line equation \(y=ax+b\), where \(a=K_s\) and \(b=\mu_{\text{max}}\) is performed.

### Warpholomeew-Gurevich Linearization

For the determination of the kinetic parameters in equation (6), the approach suggested by Gurevich-Bartholomew can also be used. In this case, the initial experimental parameters are represented by modifications of equations (5) and (6) as follows:

\[
\frac{\ln \left( \frac{X}{X_0} \right)}{\frac{1}{\tau}} - \frac{\mu_{\text{max}} S}{K_s + S} = \frac{S_j - S}{\tau_j - \tau_i} - \frac{S - S_i}{\tau - \tau_i}
\]

where: \(X_0\) - final biomass concentration, g/dm³; \(X\) - current biomass concentration, g/dm³; \(X_0\) - initial biomass concentration, g/dm³; \(S_0\) and \(S\) - initial and current substrate concentration, g/dm³.

To determine the kinetic parameters in the Monod equation, the dependence \(\frac{\ln \left( \frac{X}{X_0} \right)}{\frac{1}{\tau}} = \frac{S}{\tau_S} - k_{\text{im}}\) is used, and the kinetic parameters in the Monod model are determined from the straight line equation.

### Linearization by Substrate Consumption and Product Accumulation

Gurevich-Warpholomeew linearization approach also allows the growth rate to be determined using the changes in the substrate and the product concentration. For this purpose, equation (10) would acquire the form:

\[
\frac{\ln \left( \frac{S}{S_0} \right)}{\ln \left( \frac{X}{X_0} \right)} = \frac{\mu_{\text{max}} S}{K_s + S} - \frac{K_s}{\tau_j - \tau_i} \tag{11a}
\]

\[
\frac{\ln \left( \frac{P}{P_0} \right)}{\ln \left( \frac{X}{X_0} \right)} = \frac{\mu_{\text{max}} S}{K_s + S} - \frac{K_s}{\tau_j - \tau_i} \tag{11b}
\]

where \(S_0; S_i; S_j\) - initial substrate concentration, g/dm³; substrate concentration corresponding to \(t_i\), g/dm³; substrate concentration corresponding to \(t_j\), g/dm³; \(\tau_j - \tau_i\) - time interval equal to the difference between the final and the current process time, h; \(P_0; P_i; P_j\) - product concentration corresponding to \(t_i\), g/dm³; product concentration corresponding to \(t_j\), g/dm³; \(P_{\text{out}}\) - maximum concentration of the product, g/dm³.

The equations are plotted in the corresponding coordinates \(\ln \left( \frac{S}{S_0} \right)\) and \(\ln \left( \frac{P}{P_0} \right)\) of \(\tau_j - \tau_i\) and \(\ln \left( \frac{X}{X_0} \right)\) of \(\tau_j - \tau_i\). Parameter \(K_s\) is determined from the angular coefficient of the straight lines, while \(\mu_{\text{max}}\) is determined from the cut-off.

### Linearization Methods in the Logistic Curve Model

In this case, the biomass concentration change is described by the logistic curve equation, which implies the destruction of part of the cells during the process. This process is proportional to the square of biomass, and the proportionality factor \(\beta\) is called coefficient of internal population competition.

\[
\frac{dX}{d\tau} = \mu X - \beta X^2 \tag{12}
\]

Equation (12) can be solved analytically, and two approaches can be used for this purpose.

#### Angelov and Kostov Linearization (Kostov, 2015)

In the first approach, linearization is done assuming that \(\beta=\mu_{\text{max}}/X_0\) and \(\Delta\tau = \text{const}\). In this case, equation (12) can be represented in real form:

\[
\Psi = 1 - \frac{X(\tau)}{X(\tau + \Delta\tau)} = \left(1 - \exp \left(-\frac{\Delta\tau}{\Delta\tau}\right)\right) \left[1 - \frac{X_0}{X}\right] \tag{13}
\]

In this case, the dependence \(\Psi=\psi(X)\) is plotted, with the final biomass concentration \(X_0\) being determined from the cut-off and the angular coefficient of the line being numerically equal to \(1 - \exp \left(-\frac{\Delta\tau}{\Delta\tau}\right)\).

#### Linearization through natural logarithms (Kedia et al., 2007)

Equation (12) can be linearized by logarithmization. In this case, its appearance would be:

\[
\ln \left( \frac{X}{X_0} \right) = \mu_{\text{max}} \tau - \ln \left( \frac{X_0}{X_0} - 1 \right) \tag{14}
\]

Thus, the angular coefficient determines the specific growth rate, while the free member in the equation determines the maximum biomass concentration. Equation (14) implies only the use of the exponential phase results as otherwise the kinetic parameters of equation (12) cannot be determined.

### RESULTS AND DISCUSSION

Before proceeding to the determination of the kinetic parameters, it is necessary to consider the fermentation dynamics. The results of the two fermentation processes carried out with free and immobilized cells are summarized in Table 1.

The main fermentation process in this variant was 96 hours in the free cell fermentation and 72 hours in the immobilized cell fermentation. Immobilized cells began the fermentation more slowly, but quickly made up for their lagging behind. As a result of the higher active cell concentration in the free cell fermentation, the fermentation process was carried out faster and finished in about 240 hours, while the immobilized cells needed another 24-36 hours for the complete beer maturation. The primary amount of alcohol for both variants accumulated during the main fermentation. The main biomass amount was accumulated during the main fermentation period, and after that the yeast cells entered the stationary growth phase.
Regarding the main metabolites defining the beer characteristics, the following main dependencies were observed. Free cells accumulated higher amounts of esters than immobilized cells. For both types of fermentation, approximately 60% of the esters accumulated until the end of the main fermentation, after which a constant concentration was established. A similar trend to that of esters was observed with higher alcohols. The experimental data showed that about 15% less of these metabolites were accumulated during immobilized cell fermentation than during free cell fermentation. Similarly to the esters, the major amounts of higher alcohols accumulated until the end of the main fermentation. Immobilized cells accumulated higher concentrations of carbonyl compounds: aldehydes and vicinal diketones. The maximum for both metabolite groups was in the first 24-36 hours in the immobilized cell fermentation followed by a rapid reduction, and the beverage matured quite quickly. In the free cell fermentation, the maximum was fuzzy and was maintained throughout the entire period of the main fermentation, which also affected the longer beer maturation and the extension of the overall fermentation time.

### Table 1: Dynamics of the fermentation process for beer production with free and immobilized cells at a constant fermentation temperature of 15°C

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Biomass (g/dm³)</th>
<th>Real extract (mg/dm³)</th>
<th>Alcohol (mg/dm³)</th>
<th>Vicinal diketones (mg/dm³)</th>
<th>Aldehydes (mg/dm³)</th>
<th>Esters (mg/dm³)</th>
<th>Higher alcohols (mg/dm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.0</td>
<td>129.8</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>24</td>
<td>1.1</td>
<td>117.2</td>
<td>5.4</td>
<td>0.5</td>
<td>20.1</td>
<td>71.3</td>
<td>3.3</td>
</tr>
<tr>
<td>48</td>
<td>1.3</td>
<td>98.5</td>
<td>8.6</td>
<td>0.6</td>
<td>12.3</td>
<td>71.3</td>
<td>11.0</td>
</tr>
<tr>
<td>72</td>
<td>1.4</td>
<td>85.0</td>
<td>15.0</td>
<td>0.6</td>
<td>18.2</td>
<td>83.2</td>
<td>25.2</td>
</tr>
<tr>
<td>96</td>
<td>1.5</td>
<td>75.8</td>
<td>27.3</td>
<td>0.7</td>
<td>20.1</td>
<td>105.9</td>
<td>43.0</td>
</tr>
<tr>
<td>120</td>
<td>1.6</td>
<td>72.0</td>
<td>30.5</td>
<td>0.6</td>
<td>21.3</td>
<td>92.1</td>
<td>40.1</td>
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<td>34.4</td>
<td>0.6</td>
<td>22.3</td>
<td>88.6</td>
<td>39.1</td>
</tr>
<tr>
<td>168</td>
<td>1.7</td>
<td>60.0</td>
<td>35.0</td>
<td>0.6</td>
<td>18.1</td>
<td>130.1</td>
<td>52.2</td>
</tr>
<tr>
<td>192</td>
<td>1.8</td>
<td>54.0</td>
<td>36.7</td>
<td>0.5</td>
<td>15.6</td>
<td>175.1</td>
<td>64.4</td>
</tr>
<tr>
<td>216</td>
<td>1.8</td>
<td>53.5</td>
<td>36.7</td>
<td>0.4</td>
<td>14.2</td>
<td>150.0</td>
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</tr>
<tr>
<td>240</td>
<td>1.8</td>
<td>53.3</td>
<td>36.8</td>
<td>0.3</td>
<td>22.3</td>
<td>88.6</td>
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</tr>
<tr>
<td>264</td>
<td>1.8</td>
<td>53.2</td>
<td>36.8</td>
<td>0.2</td>
<td>14.3</td>
<td>92.2</td>
<td>58.8</td>
</tr>
</tbody>
</table>

### Determination of the kinetic parameters in the Monod equation

The results of the kinetic parameter determination in the Monod equation (equation (6)) are presented in Figure 1 and Table 2. The comparison of the model data with the experimental results is presented in Figure 3. The data on the correlation coefficients of the linearized functions by the different methods are shown in Table 2. Data on the correlation coefficients between the experimental data and the calculated biomass concentration are shown in Figure 3.

As can be seen from Table 2, the four linearization methods result in relatively close results with respect to the maximum specific growth rate $\mu_{max}$ and the saturation constant $K_S$. The only exception is the Gurevich-Wartholomeew method, where $K_S$ has a relatively low value, i.e. it can be expected that with these parameters the microbial population will grow at the highest rate.

As can be seen from Figure 3, the calculated biomass concentration undervalue the experimental data. The only exception to some extent is the Gurevich-Wartholomeew method. This is due to different and, in most cases, complex reasons. First of all, the method for the analytical determination of the kinetic parameters suggests that the culture grows on an inexhaustible substrate. In this case, the determined maximum specific biomass growth rate as well as the saturation constant refer to the exponential phase. Thus, the accuracy of the models is greatly reduced as they cannot adequately describe the lag phase and the entry into the stationary phase. Secondly, the accuracy of the model largely depends not so much on the maximum specific growth rate but on the determined saturation constant. As wort is a complex carbon source (it contains fermentable sugars, amino acids, vitamins, etc.), there are significant deviations from the theory and $K_S \approx S$ in three of the four methods, which in turn leads to a significant reduction of $\mu$. The model calculated with the parameters determined by Gurevich-Wartholomeew is the most accurate one in this case. This is due to the fact that $K_S << S_0$, and therefore the local growth rate will be as close to the maximum possible growth rate as possible.
The observed disparity to the substrate can be explained by its complexity. According to Pirt, 1975, in cultivation in complex nutrient media there are significant exceptions to the theory, and in most cases $K_s$ is within the substrate concentration.

Improvement of the accuracy of these models can be achieved by performing the calculation procedure after the lag phase (Figure 3). In free cell fermentation the lag phase was about 48 hours. During that time the biomass adapted to the new conditions and active fermentation began.
In addition to consumption – consumption 0.001167 – 0.0025 µ.

The results obtained by equations (11a) and (11b) are of considerable interest. They show that the linearization by substrate consumption has a higher accuracy than the linearization by product accumulation. In addition, they give much higher maximum specific growth rates than the results by other methods and a relatively high association between the population and the complex substrate wort. This is probably due to the fact that they describe the process in relation to the local substrate consumption rates and the product obtained. Figure 3 shows that the substrate consumption model is more accurate, since the process of alcohol fermentation is a rather substrate-dependent process.

Analogous discussion can also be made for the description of the immobilized cell process. Unlike numerical methods for modelling and kinetic determination, there is no clear picture of the difference between free and immobilized cell processes. This is due to the fact that these methods do not take into account all aspects of fermentation and therefore cannot fully appreciate the advantages and disadvantages of fermentation with immobilized cells. It can be said that the Gurevich-Wartholomeew method for determining the kinetic parameters as well as the substrate linearization have a relatively high accuracy. They are easy and fast to perform and allow the evaluation of the kinetics of microbial growth for local periods of fermentation, which is important for the relatively long beer production processes. Models in which the kinetic parameters are calculated from the local substrate and product concentrations are distinguished in immobilized cell fermentation. This is probably due to the fact that although the diffusion resistances in the immobilized cell system are not really reported in the equation system, calculations on the local substrate and product level give an idea of the diffusion in the capsules.

The data in Table 3 show that free cells grew at a higher growth rate than immobilized cells, probably due to diffusion constraints in the immobilized cell system. It is interesting that the coefficient of internal population competition in the immobilized cell system is lower, which is probably due to the high concentration of biomass provided by the immobilized cells. Equation (14) is more accurate from these two models. This is due to the fact that it includes determination of the growth rate for the whole fermentation period, whereas equation (13) requires the removal of the points comprising the lag phase and part of the stationary phase, i.e. the determination is almost only of the exponential growth phase. This explains why the model predicts faster entrance into the stationary phase and why it gives a higher specific growth rate. In addition to the cell growth, the reverse process (cell death) also occurs in the microbial population. It is interesting to assess the impact of cell death on the accuracy of kinetic parameters in the long fermentation process. The results from the identification of the parameters according to equations (13) and (14) are presented in Table 3 and in Figure 3. Generally, this method yields more accurate results than classical methods, but together these methods can reveal different aspects of the fermentation kinetics.

### Table 2: Kinetic characteristics of biomass growth determined by different linearization methods

| \( \mu_{\text{max}} \) & \( K_{\text{m}}/ \text{g/dm}^3 \) | \( \mu_{\text{max}} \) | \( K_{\text{m}}/ \text{g/dm}^3 \) |
|----------------|----------------|----------------|----------------|
| Free cells     |                |                |                |
| Linearization | R²             | R²             |                |
| Wartholomeew   | 0.000619       | 134.41         | 0.001167       | 166.63         |
| Gurevich       | 0.00117        | 173.77         | 0.0013         | 175.76         |
| Hanes          | 0.0008         | 151.86         | 0.0012         | 171.14         |
| Eadie-Hofstee  | R²             | R²             |                |
|                | 0.9609         | 0.9736         |                |

### Table 3: Kinetic characteristics - equations (13) and (14)

| \( \mu_{\text{max}} \) & \( \beta/\text{dm}^3/(g.h) \) | \( \mu_{\text{max}} \) | \( \beta/\text{dm}^3/(g.h) \) |
|----------------|----------------|----------------|----------------|
| Free cells     |                |                |                |
| Wartholomeew   | 0.0728         | 0.0413         | 0.0600         | 0.03369        |
| Gurevich       | 0.09109        |                | 0.785          |
| Through natural logarithms | 0.0224 | 0.012687 | 0.0158 | 0.00886 |
| R²             | 0.8581         | 0.985          |                |

The results obtained by equations (13) and (14) are of considerable interest. They show that the linearization by substrate consumption has a higher accuracy than the linearization by product accumulation. In addition, they give much higher maximum specific growth rates than the results by other methods and a relatively high association between the population and the complex substrate wort. This is probably due to the fact that they describe the process in relation to the local substrate consumption rates and the product obtained. Figure 3 shows that the substrate consumption model is more accurate, since the process of alcohol fermentation is a rather substrate-dependent process.

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| \( \mu_{\text{max}} \) & \( \beta/\text{dm}^3/(g.h) \) | \( \mu_{\text{max}} \) | \( \beta/\text{dm}^3/(g.h) \) |
|----------------|----------------|----------------|----------------|
| Free cells     |                |                |                |
| Wartholomeew   | 0.0728         | 0.0413         | 0.0600         | 0.03369        |
| Gurevich       | 0.09109        |                | 0.785          |
| Through natural logarithms | 0.0224 | 0.012687 | 0.0158 | 0.00886 |
| R²             | 0.8581         | 0.985          |                |
CONCLUSION

Eight different methods for analytical identification of the kinetic parameters for yeast biomass growth in beer production have been examined in the present paper. Higher precision has been found to be provided by methods that involve identification by local substrate consumption rates and / or product accumulation, as well as those involving calculations over the entire fermentation period. The results obtained show that in future experiments and modelling the kinetic parameters have to be calculated using the Gurevich-Varfolomeev method, substrate consumption and product assimilation models and by solving the logistic curve by linearization through natural logarithms.

REFERENCES


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