

MODELING OF ALCOHOL FERMENTATION IN BREWING – CARBONYL COMPOUNDS SYNTHESIS AND REDUCTION

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ABSTRACT

A mathematical model was developed for studying the carbonyl compounds synthesis and reduction in beer fermentation with alginate-chitosan microcapsules with liquid core. The model was based on the results for the influence of the fermentation temperature, original wort gravity and immobilized cells mass on the carbonyl compounds synthesis and reduction. The obtained model described with high accuracy the vicinal diketones synthesis and reduction and confirmed the experimental data. However, the model was not in agreement with the data for aldehydes synthesis and reduction. It did not take into account the second peak in aldehyde concentration during maturation. It can be assumed that the peak was related to maltotriose uptake by the used yeast strain. Nevertheless, the obtained model can be used for the description of carbonyl compounds synthesis and reduction in beer fermentation with immobilized cells.

INTRODUCTION

In brewing, the fermentation is one of the longest stages as well as an important aromatic compound production step. Indeed, fermentation has the main impact on process productivity and product quality. The brewing process productivity can be increased by the introduction of immobilized cells technology. It allows beer production to be accomplished in as little as 2-3 days (Branyik et al, 2005).

Yeast metabolism during fermentation and maturation affects significantly beer flavor. Ethanol, CO₂, esters and fusel alcohols have positive contributions to beer flavor. Dimethyl sulphide, hydrogen sulphide, and carbonyl compounds contribute to beer flavor defects (Meilgaard, 1975).

Carbonyl compounds are important because they have a high flavor potential and a significant influence on the

flavor stability of beer. Over 200 carbonyl compounds have been detected in beer (Russell, 2006). The most important carbonyls are acetaldehyde and VDK. Acetaldehyde has unpleasant “grassy” flavor and aroma. It is of special interest because of its role as the immediate precursor of ethanol. VDK – diacetyl (2,3-butanedione) and 2,3-pentanedione have “butterscotch” and “toffee” aroma and taste (Briggs et al., 2004). The VDK concentrations in beer determined the maturation process time (Wilaert, 2007).

The aim of this work was to develop a mathematical model of the carbonyl compounds synthesis and reduction in beer fermentation with immobilized yeast. The model parameters identification was based on experimental data for the effect of T_{MF}, OE and Mic on the synthesis and reduction of VDK and aldehydes in beer produced under laboratory conditions.

MICROORGANISMS AND FERMENTATION CONDITIONS

The fermentations were carried out with bottom-fermenting yeast strain *Saccharomyces pastorianus* Saflager S-23. Immobilization procedure was previously reported in (Parcunev et al., 2012). The fermentations (main and secondary) were carried out with 400 cm³ sterile wort in fermentation bottles equipped with airlocks. The fermentation conditions are shown in Table 1. The data was part of planned experiment schedule which was reported in (Naydenova et al., 2014). The maturation temperature was 4°C higher than the T_{MF}. Maturation started when the difference between the attenuation limit and apparent attenuation was approximately 20% (Naydenova et al, 2014). 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 aldehyde concentrations were determined according to (Marinov, 2010). Biomass concentration in immobilized cells was determined according to the mathematical model proposed in (Parcunev et al., 2012).

PARAMETERS IDENTIFICATION

The fermentation process kinetics was described with ordinary differential equations system (1).

$$\begin{aligned} \frac{dX}{dt} &= \mu(t, T) X(t, T) \\ \frac{dP}{dt} &= q(t, T) X(t, T) \\ \frac{dS}{dt} &= -\frac{1}{Y_{x/s}} \frac{dX}{dt} - \frac{1}{Y_{p/s}} \frac{dP}{dt} \\ \frac{dVDK}{dt} &= Y_{VDK} \cdot \mu(t, T) \cdot X(t, T) - k_{X, VDK} \cdot VDK(t, T) \cdot X(t, T) \\ \frac{dA}{dt} &= Y_A \mu(t, T) X(t, T) - k_A A(t, T) X(t, T) \end{aligned} \quad (1)$$

$$\mu = \mu_{\max} \frac{S}{K_{sx} + S}; \quad q = q_{p \max} \frac{S}{K_{sp} + S}$$

The parameters identification was made by software programs in MatLab Environment (Kostov et al., 2012; Mitev and Popova, 1995; Popova 1997). The software minimized the sum of squared of difference between the model outputs and experimental data with respect of models parameters:

$$E(r) = (X(k_1, k_2, \dots, k_n) - X^e)^2 + (S(k_1, k_2, \dots, k_n) - S^e)^2 + (P(k_1, k_2, \dots, k_n) - P^e)^2 + (VDK(k_1, k_2, \dots, k_n) - VDK^e)^2 + (A(k_1, k_2, \dots, k_n) - A^e)^2 \quad (2)$$

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

$$dk_i / dt = 0 \quad (3)$$

was added to the ordinary differential equations model because k_i , $i=1 \div n$ was constant. For solving the overall differential equations system based on the Runge-Kutta formula of 4-5 order was used MATLAB function “ode45”. All parameters are shown in table 3.

CARBONYL COMPOUNDS SYNTHESIS AND REDUCTION

Vicinal diketones

Diacetyl and 2,3 - pentanedione are produced by yeast during fermentation. Diacetyl is the more important substance because of its lower flavor threshold. Both VDK are formed from intermediates of the amino acid biosynthesis. Diacetyl relates to valine and 2,3-pentanedione relates to isoleucine. The first intermediates in this metabolism are α -acetylactate and α -acetohydroxybutyrate. These components are discharged from the cell and undergo an oxidative decarboxylation to form diacetyl and 2,3-pentanedione. Yeast takes in these substances again and reduces them to 2,3-butanediol and 2,3-pentanediol, respectively. Owing to their high threshold, both resulting components show little influence on flavor (Handbook of brewing: Processes, Technology, Markets 2009; Debourg, 1999). The formation of the diketones is illustrated in Figure1.

Table 1
Fermentation conditions for beer production with immobilized cells

№	T _{MF}	T _{MATF}	OE	M _{IC}
-	°C	°C	% w/w	g
1	10	14	10.5	5
2	10	14	10.5	15
3	12.5	16.5	13	10
4	12.5	16.5	8.5	10
5	15	19	10.5	5
6	15	19	10.5	15
7	17	21	13	10

Aldehydes synthesis and reduction

Several aldehydes arise during wort production; others are formed as intermediates in the biosynthesis of higher alcohols from oxo-acids by yeasts (Briggs et al., 2004). Acetaldehyde synthesis is linked to yeast growth. Its concentration is maximal at the end of the growth phase, and is reduced at the end of the primary fermentation and during maturation by the yeast cells (Willaert, 2007). Removal of acetaldehyde is favored by increased yeast content during maturation (Russell, 2006)

MATHEMATICAL MODELS AND THEIR EXPLANATION

The fermentation with immobilized cells can be described with the equations for batch fermentation with free cells as previously reported (Parcunev et al., 2012; Vassilev et al., 2013). These are the first three equations in (1). For the adequate model development it is necessary to take into account some steps in the metabolites synthesis and reduction during beer fermentation.

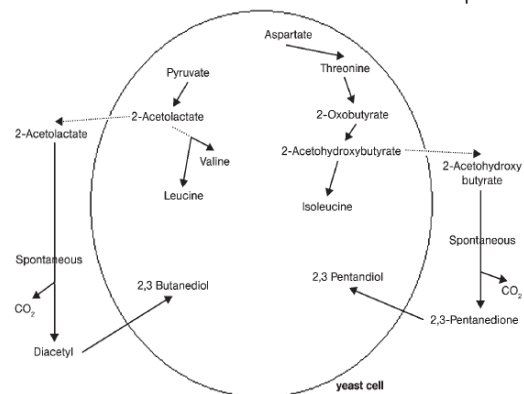


Figure 1. Formation and reduction of vicinal diketones
(Handbook of brewing: Processes, Technology, Markets 2009)

The VDK synthesis is a result of sugars and amino acids uptake by yeast for α -acetylactate and α -acetohydroxybutyrate production. Therefore, these two components have to be considered in the model. On the other hand, the sugars and amino acids uptake is associated with yeast growth. Thus, the VDK synthesis

is associated with cell growth. It can be taken into account in the model by a yield coefficient Y_{VDK} .

VDK reduction has two stages – chemical and biological. The first phase is the chemical conversion of α -acetolactate and α -acetohydroxybutyrate to diacetyl and 2,3-pentanedione, respectively. It can be intensified with the increase in temperature during maturation. The biological stage includes the uptake of diacetyl and 2,3-pentanedione by yeasts and their reduction to acetoin and 2,3-pentanediol, respectively. This phase can also be intensified with the increase in temperature. In our experiments the maturation temperature was 4 °C higher than the main fermentation temperature, which resulted in faster VDK reduction. VDK synthesis and reduction can be described with the fourth equation in the system (1) after considering all the factors that affect it.

In our previous study (Vassilev et al., 2013) it was shown that the aldehyde synthesis was associated with yeast growth, and their reduction - with the biomass concentration in the bioreactor and the aldehydes concentration in fermenting beer. These dependencies are presented in the fifth equation of the differential equations system (1).

According to differential equations system (1) the kinetic parameters depended on the fermentation temperature.

In the works of Andreas-Toro et. al, 1998 and Ramirez and Maciejowski, 2007 was found that the kinetic parameters and especially the specific growth rate could be described with an equation similar to the Arrhenius equation:

$$\mu_i = \mu_{i_{max}} \exp\left(-\frac{E_{\mu i}}{RT}\right) \quad (4)$$

Therefore, it can be suggested that the specific growth rate is a function of the cultivation conditions. Such kind of models are developed for the processes with free cells, but the diffusion resistances in the processes with immobilized cells may lead to some differences, which have to be considered. Thus, for simplification, the initial studies were made by the mathematical equations system and Monod equation (1). In the present work $E_{\mu i}$ was not evaluated. Ramirez and Maciejowski, 2007 showed that $E_{\mu i}$ ranged between -68.4 and 211.9 kcal/gmole. The value depended on the following parameters: the specific growth rate, specific substrate consumption rate or the specific metabolites production rate.

RESULTS OF FERMENTATIONS AND KINETIC PARAMETERS

Figure 2 and Figure 3 present the fermentation dynamics for one of the investigated variants (variant 3 of Table 1 and variant 7 of Table 1, respectively) as well as the comparison between the mathematical model (1) and the experimental data. The other variants showed similar fermentation dynamics.

The primary fermentation time and maturation time of the studied variants are presented in Table 2. It can be

found that the increase in the T_{MF} (the maturation temperature, respectively) led to the fermentation time reduction. It has to be noted that the observed trend deviation in the variant 7 was due to the fermentation kinetics.

The kinetic parameters of the studied fermentations are presented in Table 3. The results confirmed our previous observations (Parcunev et al., 2012; Vassilev et al., 2013) that the immobilization did not significantly affect the primary metabolism of immobilized yeast. The kinetic parameters indicated high specific fermentation rate (dX/dt and dP/dt), which decreased with the increase in OE due to substrate inhibition and catabolite repression. The maximum specific ethanol production rate varied between 0.48 and 1.19 g/(g.h) depending on the operational conditions. The major amount of the ethanol was produced by the end of the main fermentation because 80% of fermentable sugars were fermented during the primary fermentation.

Table 2

Fermentation time of experimental variants

(according Naydenova et al, 2014)

№	Time _{MF}	Time _{MATF}	Time
-	h		
1	288	168	456
2	192	204	456
3	204	180	294
4	108	156	264
5	144	96	240
6	78	156	234
7	120	172	292

The obtained results for the VDK synthesis and reduction were very interesting. The increase in T_{MF} resulted in an increase in the average VDK synthesis rate ($\mu \cdot X \cdot Y_{VDK}$). The most interesting results were recorded during the main fermentation at highest temperature (variant 7). The results obtained did not confirm the suggestion that the yield coefficient Y_{VDK} would be very high. It can be explained by the simultaneous VDK synthesis and reduction at 17 °C. M_{IC} increase affected contradictory the VDK synthesis. It should be noted that the M_{IC} increase led to an increase in yield coefficient Y_{VDK} at 10°C (variants 1 and 2). On the contrary, at 15 °C the M_{IC} increase resulted in decreased yield coefficient Y_{VDK} (variants 5 and 6). It can be assumed that the combination of high T_{MF} and M_{IC} caused accelerated VDK reduction, which took place simultaneously with VDK synthesis. At constant T_{MF} and M_{IC} the OE increase led to decrease in Y_{VDK} (variants 3 and 4).

The specific VDK reduction rate depends on the local VDK concentration and the biomass concentration. However, the increase in temperature and biomass concentration resulted in accelerated VDK reduction. Unfortunately, the VDK synthesis and reduction rates in the microcapsules could not be determined, because it was difficult to measure the VDK concentration in the capsule.

Figure 4 shows the average VDK reduction rate by the yeasts in stationary growth phase. It was calculated by the multiplication of VDK reduction coefficient ($K_{X,VDK}$) and the biomass concentration in stationary growth phase ($X(stat)$). It can be found that there was a region with optimal operational conditions for carrying out maturation – OE=10-13 °P and maturation temperature 14-19 °C. Therefore, the optimal

fermentation conditions were OE=10-13 °P and T_{MF} = 10-15°C. This coincides with the optimal interval for fermentation using bottom-fermenting yeast stains. The increase in temperature resulted in deterioration in beer quality. The VDK reduction rate at temperatures above 21 °C was not investigated because these temperatures were not proper for lager beer maturation.

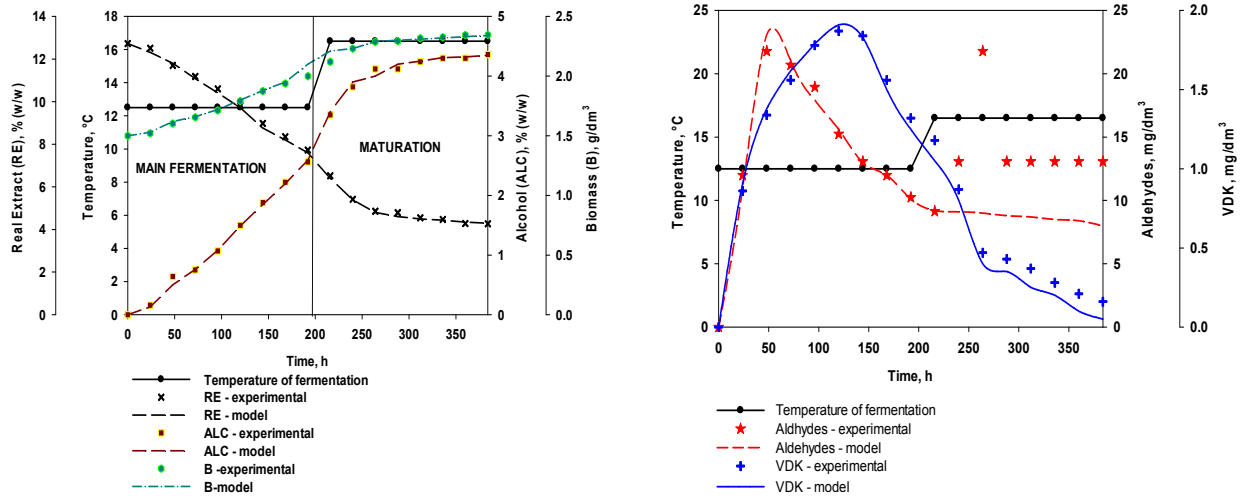


Fig.2. Fermentation dynamics(var. 3, tabl.1)

* experimental results according to Naydenova et al, 2014

Table 3

Kinetic parameters of the alcoholic fermentation and the carbonyl compounds synthesis and reduction

No	μ_{max} h ⁻¹	K_{SX} g/dm ³	q_{pmax} g/(g,h)	K_{SP} g/dm ³	$Y_{X/S}$ -	$Y_{P/S}$ -	Y_{VDK} mg/(g,h)	$K_{X,VDK}$ mg/(g,h)	Y_A mg/(g,h)	K_A mg/(g,h)	E(r)
1	0.158	229.5	1.19	229.5	0.148	13.83	2.80	0.012	39.1	0.0056	7.73
2	0.124	248.6	0.49	209.8	0.229	44.42	3.47	0.048	26.7	0.0011	5.57
3	0.421	240.5	0.53	228.2	0.051	28.42	5.34	0.091	39.8	0.0012	8.71
4	0.493	224.7	0.96	216.9	0.151	7.155	6.63	0.087	69.5	0.009	10.1
5	0.195	256.4	0.48	246.8	0.310	4.42	7.23	0.026	98.2	0.0226	4.3
6	0.278	241.2	0.51	246.7	0.13	5.81	4.48	0.025	100.3	0.125	5.2
7	0.387	245.6	1.09	249.6	0.015	6.21	4.54	0.022	105.2	0.109	10.2

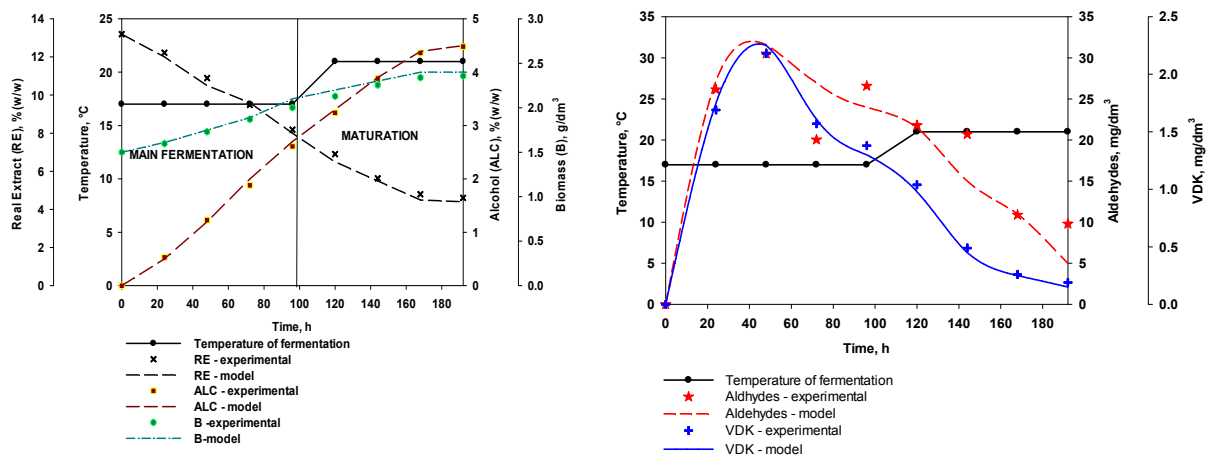


Fig.3. Fermentation dynamics(var. 7, tabl.1)

* experimental results according to Naydenova et al, 2014

It has to be noted that the different VDK synthesis rates caused the VDK maximums to be determined at different phases of main fermentation. At low T_{MF} and M_{IC} the maximum concentration was detected at the end of main fermentation. The increase in T_{MF} and M_{IC} resulted in VDK peaks at the beginning of the main fermentation (1-3 days). The data confirmed the observations in Naydenova et al., 2014.

The model showed almost complete diacetyl and 2,3-pentanedione reduction. Nevertheless, the VDK concentration in beer produced with immobilized yeast was higher than the VDK concentration in conventional beer.

Interesting trends were observed for the aldehyde synthesis and reduction (Table 3). The increase in T_{MF} , led to the accelerated aldehydes synthesis. It is interesting to note that the T_{MF} increase with 5 °C caused almost 3-fold increase in the yield coefficient Y_A . It can be hypothesized that it is due to a fast cell growth irrespective of the immobilization. At constant T_{MF} and M_{IC} the OE increase led to decrease in the yield coefficient Y_A (variants 3 and 4).

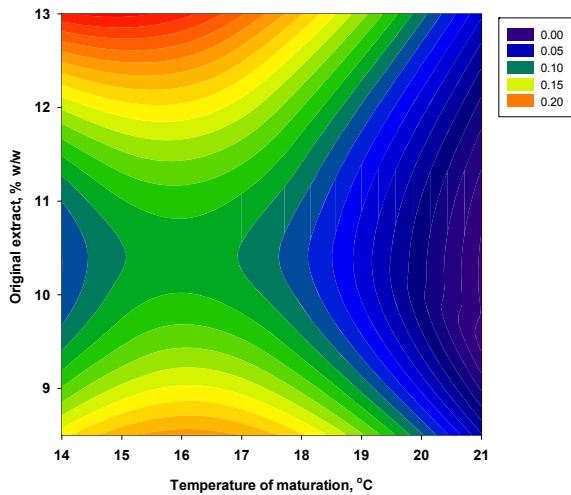


Fig.4. Average VDK reduction rate by biomass in a stationary growth phase ($K_{X,VDK} \cdot X(\text{stat})$)

The specific aldehydes reduction rate ($k_a \cdot A \cdot X$) was relatively constant. The main reason was a phenomenon that was not taken into account by the mathematical model. For all the variants a second peak of aldehydes was observed at the beginning of the maturation. It can be assumed that this was due to the maltotriose uptake. Maltotriose is utilized only in the later stages of alcoholic fermentation, which probably caused new aldehydes synthesis.

The M_{IC} increase and the similar specific aldehydes reduction rate led to an increase in the average aldehydes reduction rate.

The results in Table 2 correspond to the observed fermentation dynamics. The OE increase resulted in longer fermentation time. The increase in M_{IC} and T_{MF} led to a reduction in the primary fermentation time. It should be highlighted that the temperature affected most

significantly the maturation time reduction. On the contrary, the M_{IC} increase was related to the synthesis of more carbonyl compounds, which caused prolonged maturation.

CONCLUSION

A detailed study of the fermentation kinetics and the carbonyl compounds synthesis and reduction in beer fermentation with immobilized cells was carried out. The results showed that the carbonyl compounds kinetics affected significantly on the fermentation time. The carbonyl compounds kinetics was a function of T_{MF} (maturation temperature, respectively), M_{IC} and OE. The results showed that the temperature affected most significantly the carbonyl compounds synthesis and reduction. The M_{IC} increase led to the synthesis of more carbonyl compounds, which caused prolonged maturation. The increase in the wort extract resulted in longer fermentation time.

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LIST OF SYMBOLS

- T_{MF} – main fermentation temperature, °C
- OE – original wort gravity, °P
- M_{IC} – immobilized cells mass, g
- $X(t)$ – biomass concentration, g/dm³;
- $P(t)$ – ethanol concentration, g/dm³;
- $S(t)$ – substrate (extract) concentration, g/dm³;
- μ – specific biomass growth rate, h⁻¹;
- μ_{max} – maximal specific biomass growth rate, h⁻¹;
- q_p – specific ethanol production rate, g/(g.h);
- q_{pmax} – maximum specific ethanol production rate, g/(g.h);
- K_{SX} – Monod constant for the substrate, g/dm³;
- K_{SP} – Monod constant for the product, g/dm³;
- A – aldehydes concentration, mg/dm³;
- Y_A – yield coefficient for aldehydes, mg/(g.h);
- k_A – reduction coefficient for aldehydes, mg/(g.h);
- VDK – vicinal diketones concentration, mg/dm³;
- Y_{VDK} – yield coefficient for vicinal diketones, mg/(g.h);
- $K_{X,VDK}$ – reduction coefficient for vicinal diketones, mg/(g.d);
- $E(t)$ – error between the experimental and model data;
- R – universal gas constant, J/(kmol.K);
- T – absolute temperature, K;
- E – activation energy, J/mol;
- MF – main fermentation;
- MatF – maturation.

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