MODELING OF ALCOHOL FERMENTATION IN BREWING – COMPARATIVE ASSESSMENT OF FLAVOR PROFILE OF BEERS PRODUCED WITH FREE AND IMMOBILIZED CELLS

Stoyan Vassilev, Vessela Naydenova, Mariana Badova, Vasil Iliev, Maria Kaneva, Georgi Kostov
Department "Technology of wine and brewing"
University of Food Technologies
4002, 26 Maritza boulvd., Plovdiv, Bulgaria
E-mail: george_kostov2@abv.bg; vesi_nevelinova@abv.bg; m_kaneva@abv.bg; stoyanvassilev5@gmail.com; mariqna_badova@abv.bg; vasil_iliev_1988@abv.bg;

Silviya Popova
Institute of System Engineering and Robotics
Bulgarian Academy of Sciences
Acad. G. Bonchev str. bl.2, Sofia-1113, Bulgaria
E-mail: popova_silvia2000@yahoo.com

KEYWORDS

modeling, kinetics, flavor profile, esters, aldehydes, higher alcohols, metabolism

ABSTRACT

A detailed investigation of bioengineering constants for the accumulation of major yeast metabolites in beer produced by free and immobilized cells was carried out. The kinetic constants were used to determine the effect of immobilization on yeast metabolism. The esters synthesis dynamics was similar for the free and the immobilized cell which was confirmed by the kinetic model. The dynamics of the higher alcohol synthesis was comparable for the free and immobilized cell, but there were some differences. The dynamics of the higher alcohols accumulation for the free and immobilized cells was similar, but there were some differences between the two used strains. These differences were due to the influence of mass transfer between the liquid and the capsules, which reflected mostly in the metabolism of some amino acids. There were significant differences in the aldehyde synthesis and reduction by immobilized and free cells. The free cells of both yeast strains showed distinct maximum of aldehydes, where after the aldehydes reduction started. On the other hand, the aldehyde peaks were not so distinct for the immobilized cells and the aldehydes concentration was relatively constant during fermentation. The explanation for this difference can be found in the kinetic parameters. The model described with high accuracy the beer fermentations with immobilized and free cells and confirmed the experimental data. The obtained data would be used for developing of control strategy fermentation process to obtain beverages with different organoleptic profile.

INTRODUCTION

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. 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).

Yeast metabolism during fermentation and maturation affects significantly on beer flavor. Ethanol, CO_2, esters and fusel alcohols have positive contributions to beer flavor. Dimethyl sulphide and hydrogen sulphide, diacetyl, and aldehydes contribute to 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-products optimization in certain limits. Fermentation and maturation 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. In such a competitive market, the potential time savings, proposed 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). 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 aim of this work is to determine the influence of immobilization on the yeast metabolism using mathematical models. The dynamics of the main yeast metabolites - ethanol and CO_2, the biomass concentration and some of secondary yeast metabolites - esters, aldehydes and higher alcohols were studied. The differences between fermentations with immobilized and free cells were determined by the developed mathematical models for yeast metabolites.

MICROORGANISMS AND FERMENTATION CONDITIONS

The fermentations were carried out with top-fermenting yeast strain *Saccharomyces cerevisiae* S-33 and bottom-fermenting yeast strain *Saccharomyces pastorianus* S-23. 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.

Immobilization procedure and 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 intervals of time (10 days) to determine the impact of immobilization on the yeast metabolism. Although the fermentation with immobilized cells was faster than the one with free cells, the metabolites accumulation in the fermenting medium was influenced by diffusion limitations.

Biomass concentration of immobilized cells was determined according to mathematical model proposed in (Parcunev et.al. 2012). The concentrations of yeast metabolites – aldehydes, esters and higher alcohols were measured according to (Marinov, 2010). Because of the limited volume of wort, the analyzes were performed on the 1st, 3rd, 5th, 7th and 9th day for the fermentation with top-fermenting yeast and on the 2nd, 4th, 6th, 8th and 10th day for the fermentation with bottom fermenting yeast.

MATHEMATICAL MODELS AND THEIR EXPLANATION

Equation of fermentation

The fermentation with immobilized cells can be described with the equations for batch fermentation with free cells as previously reported (Parcunev et.al. 2012):

$$\left| \begin{array}{l} \dfrac{dX}{dt} = \mu(t)X(t) \\ \dfrac{dP}{dt} = q(t)X(t) \\ \dfrac{dS}{dt} = -\dfrac{1}{Y_{x/s}}\dfrac{dX}{dt} - \dfrac{1}{Y_{p/s}}\dfrac{dP}{dt} \end{array} \right. \tag{1}$$

The values of parameters have to be corrected with efficiency coefficients:

$$\eta_\mu = \frac{\mu_{max}^{imm}}{\mu_{max}^{free}}; \ \eta_q = \frac{q_{max}^{imm}}{q_{max}^{free}} \tag{2}$$

In our previous study (Parcunev et.al. 2012) three fermentation models were investigated, but the Monod model showed highest accuracy for description of

fermentation with immobilized and free cells. Therefore, it was chosen for further modeling of the fermentation processes:

$$\mu = \mu_{max}\frac{S}{K_{sx}+S}; \ q = q_{pmax}\frac{S}{K_{sp}+S} \tag{3}$$

The Monod equation provides a good basis for the management of the fermentation. However, it gives no information about the concentration of yeast by-products in beer and the influence of immobilization on the beer flavor profile.

Models of beer flavor profile

Beer flavor profile is formed from a large number of compounds. It is impossible to be made a mathematical model which includes the all flavor-active compounds. Therefore, three important groups of them will be used for the model of our work - higher alcohols, esters, and aldehydes. The other important flavor-active group of compounds – vicinal diketones will be discussed in next papers because of their specific synthesis and reduction. The basis of our mathematical model will be the microbiological aspects of yeast by-products synthesis and the work of (Ramirez and Maciejowski, 2007). In their paper, an algorithm for the fermentation control was made. It was based on the various substrates uptake and the synthesis of different groups of metabolites. The application of their model for the purpose of our work requires certain simplifications in the model. On the other hand, the model has to take into account the knowledge of biochemical and microbiological characteristics of processes.

Higher alcohols synthesis

More than 40 higher alcohols have been identified in beers (Engan, 1981). The flavor impressions reach from flowery to solvent-like and alcoholic. Higher alcohols are the precursors for more flavor active esters (Briggs et al., 2004). Higher alcohols can be synthesized via two routes: "*de novo*" from wort carbohydrates (the anabolic route) or as by-products of amino acid assimilation (the catabolic route) (Rusell, 2006). At the ends of primary fermentation over 90% of the higher alcohols have been built, the rest arise during the maturation process (Handbook of brewing: Processes, Technology, Markets 2009). Therefore, the higher alcohols synthesis may be associated with the biomass growth and can be described by equation (4):

$$\frac{dFA}{dt} = Y_{FA}.\mu.X(t) \tag{4}$$

Ester synthesis

Esters comprise possibly the most important set of flavor-active beer components which arise as a result of yeast metabolism. In the region of 100 distinct esters have been identified in beers (Boulton and Quain, 2001). They are formed by the intracellular reaction between a fatty acyl-coenzyme A and an alcohol (Willaert and Nedovic, 2006). Esters are produced by yeast cells during the exponential phase (60%) and the stationary phase (40%). Ethyl esters are present in the highest quantity presumably because ethanol is present in large amounts (Rusell, 2006). As mentioned above,

most of the higher alcohols are formed during primary fermentation and, since these compounds are required for ester synthesis, ester synthesis is delayed. Therefore, a significant amount of esters is built when yeast growth declines (Handbook of brewing: Processes, Technology, Markets 2009). This means that the esters synthesis may be associated with cell growth and their change can be described by equation (5):

$$\frac{dE}{dt} = Y_E . \mu . X(t) \qquad (5)$$

Carbonyls (aldehydes) synthesis and reduction

Some 200 carbonyl compounds have been detected in beers of importance to beer flavor and aroma and influenced by yeast metabolism are acetaldehyde and several other aldehydes and vicinal diketones (Boulton and Quain, 2001). Several aldehydes arise during wort production, others are formed as intermediates in the biosynthesis of higher alcohols from oxo-acids by yeasts. Acetaldehyde is of special interest as an immediate precursor of ethanol (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 (Rusell, 2006). Therefore, it must be highlighted that the aldehyde synthesis is associated with yeast growth, and their reduction - with the biomass concentration in the bioreactor and the aldehydes concentration in fermenting beer (6):

$$\frac{dA}{dt} = Y_A . \mu . X(t) - k_A . A . X \qquad (6)$$

Vicinal diketones are very important for beer flavor. Their synthesis is also linked to yeast growth. Unlike aldehydes, their reduction has both chemical and biochemical stages and therefore they will not be discussed in this paper. However, they will be mentioned in the analysis of the results.

As shown in the equations (4), (5) and (6) the synthesis of key metabolites is linked to yeast growth and can be determined by yield coefficients - Y_{FA}, Y_E, and Y_A. The aldehydes reduction can be described by k_A and the current concentrations of yeast and aldehydes in beer.

Therefore, the obtained mathematical model includes equations (1), (3), (4), (5) and (6) for the fermentation with free cells and equations (1), (2), (3), (4), (5) and (6) for the fermentation with immobilized cells

RESULTS OF FERMENTATIONS. BASIC KINETIC PARAMETERS.

The results for the yeast metabolites accumulation of yeast strain *Saccharomyces cerevisiae S-23* are shown in Figure 1. Figure 2 presented the results for yeast by-product accumulation of yeast strain *Saccharomyces pastorianus S-33*. The results are the average of three independent fermentation processes. The fermentation process kinetics was described with the ordinary differential equation (1 to 6). The identification of

parameters 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 errors of the model outputs with respect to the experimental data:

$$E(r) = (X(k_1,k_2,...,k_n) - X^e)^2 + (S(k_1,k_2,...,k_n) - S^e)^2 + (P(k_1,k_2,...,k_n) - P^e)^2 + $$
$$+ (E(k_1,k_2,...,k_n) - E^e)^2 + (FA(k_1,k_2,...,k_n) - FA^e)^2 + (A(k_1,k_2,...,k_n) - A^e)^2$$

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

$$dk_i / dt = 0$$

were added to the ordinary differential equations model because k_i, i=1÷n were constant. For solving the overall differential equations system based on the explicit Runge-Kutta of 4-5 order formula using MATLAB function "ode45". All parameters are shown on tables 1. Table 2 presents the efficiency coefficients, which indicate the influence of the immobilization on the kinetic parameters. Efficiency coefficients are the ratio of parameters of fermentation with the immobilized cells to the same parameter of fermentation with free cells.

Table 1

Kinetic parameters of metabolites accumulation in beer during fermentations with free and immobilized cells
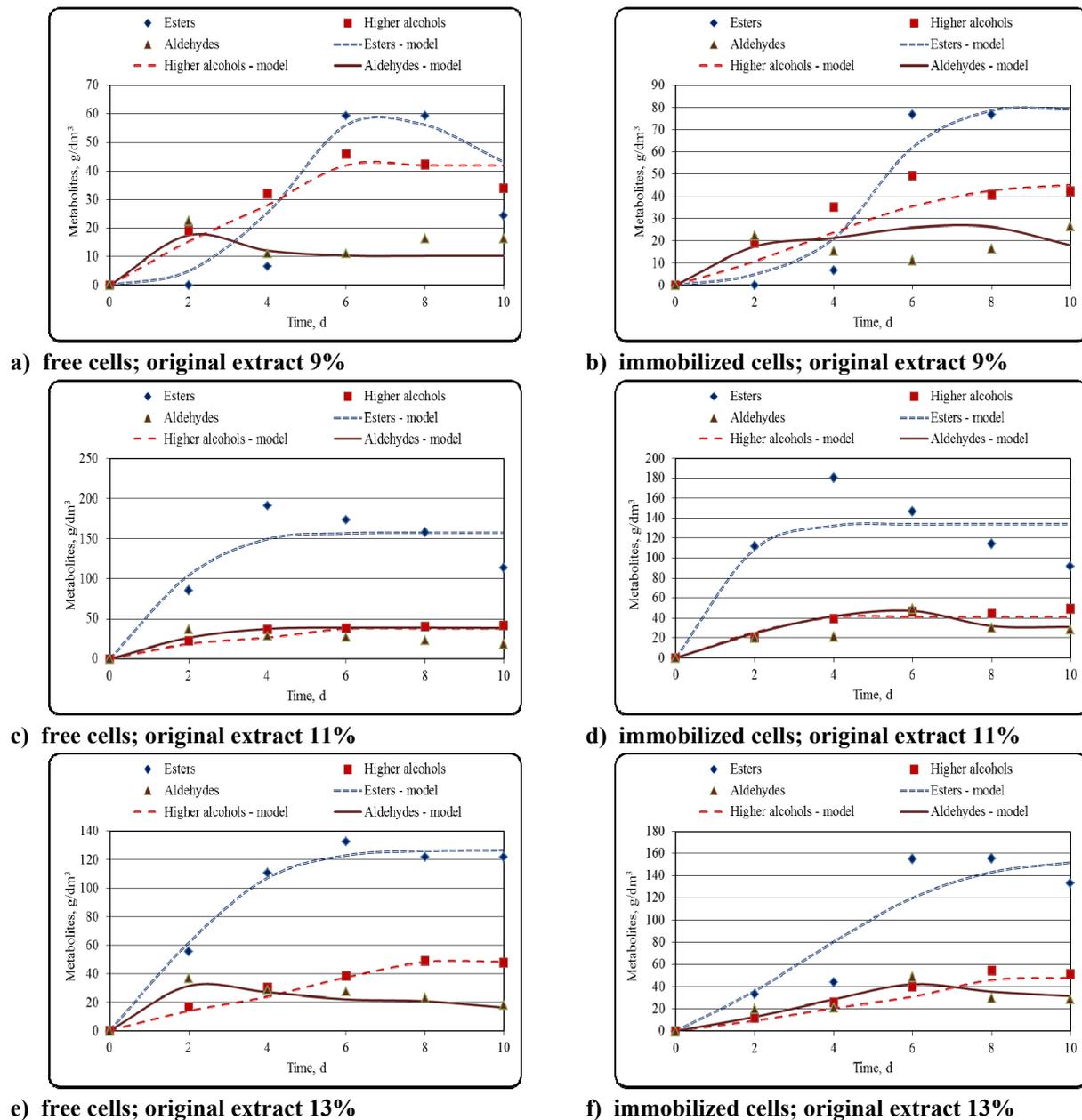
Original extract	Y_E	Y_{FA}	Y_A	K_A	Error
	mg/(g.d)				-
Saccharomyces pastorianus S-23					
9% F	12.23	12.55	7.63	0.023	**0.259**
9% IMM	13.03	4.68	16.06	0.0205	**0.345**
11% F	116.4	20.47	29.42	0.01	**0.429**
11% IMM	144.2	33.6	45.94	0.035	**0.573**
13% F	61.05	13.61	21.19	0.025	**0.129**
13% IMM	31.72	8.15	11.36	0.0513	**0.234**
Saccharomyces cerevisiae S-33					
9% F	31.99	18.8	120.1	0.0345	**0.462**
9% IMM	41.75	17.36	79.81	0.046	**0.346**
11% F	34.45	11.31	15.45	0.045	**0.547**
11% IMM	21.68	3.64	6.52	0.0678	**0.247**
13% F	184.36	19.52	51.01	0.0456	**0.101**
13% IMM	152.43	23.44	59.82	0.081	**0.246**

It was found in (Parcunev et.al. 2012) that the fermentation with immobilized cells was characterized with higher specific rates. They resulted in reduced primary fermentation time, but not in reduced total fermentation time. The total fermentation time was depended on the time for aldehydes and vicinal diketones reduction (maturation time). Therefore, the changes in the metabolism after primary fermentation were essential for the formation of a product with high quality. In terms of the mathematical model this feature should be considered. Therefore, the secondary metabolites accumulation by immobilized cells should be considered under the similar conditions with free cells for the complete evaluation of the advantages and disadvantages of the immobilized cells systems.

The modeling data (Figure 1, Table 1 and Table 2) showed no significant differences in the metabolism of free and immobilized cells of yeast strain *Saccharomyces pastorianus S-23*. The developed mathematical model describes with high accuracy the experimental data. It indicates the typical process dynamics to a large degree. However, there are also some problematic areas in the model which need to be taken into account when the model is used.

The modeling data (Figure 1, Table 1 and Table 2) showed no significant differences in the metabolism of free and immobilized cells of yeast strain *Saccharomyces cerevisiae S-33*. Similarly, the proposed model described with high accuracy the experimental data, but the similar problems as with yeast strain S-23 were observed.

These problems are connected with the microbiological aspects of fermentation and some physicochemical processes during the fermentation which have to be taken into account in the modeling. The problems will be discussed in groups, because the mathematical model was created in the same way.

a) free cells; original extract 9%

b) immobilized cells; original extract 9%

c) free cells; original extract 11%

d) immobilized cells; original extract 11%

e) free cells; original extract 13%

f) immobilized cells; original extract 13%

Figure 1 Experimental data and mathematical models for esters, higher alcohols and aldehydes during beer fermentation with free and immobilized cells of yeast strain Saccharomyces pastorianus S-23

Esters
The esters synthesis dynamics was similar for the free and the immobilized cells of yeast strain *S. cerevisiae S-33*. It should be mentioned that the immobilized cells accumulated more esters than free cells at low original wort extracts - 9% and 11%. The increase in original wort extract resulted in increased ester concentration for free cells than immobilized ones. The immobilized cells of yeast strain *S. pastorianus S-23* produced more esters than free cells. The amount of esters increased with the

increase of original wort extract. The mathematical models for both strains confirmed the experimental data. It should be highlighted that the immobilized cells accumulated a little more esters at lower original wort extracts. It can be explained that immobilization induces the inhibition of fatty acid synthesis, resulting in an accumulation of acyl-CoA that together with high levels of ethanol in immobilized cell systems enhance ethyl acetate formation (Shen et al., 2003). Furthermore, higher original wort extract limits oxygen solubility in wort, which also leads to increased ester synthesis (Dufour et al., 2003).
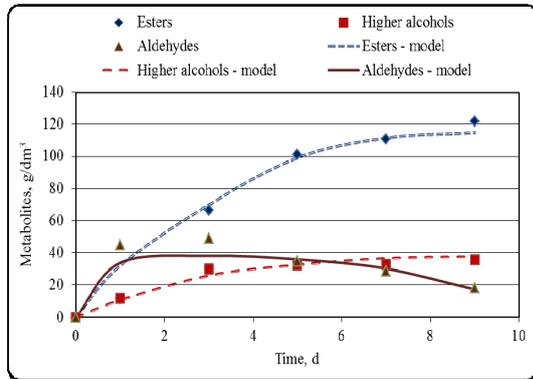
The major disadvantage of the mathematical model is that it does not take into account the chemical reduction (hydrolysis) of esters due to some physicochemical processes.
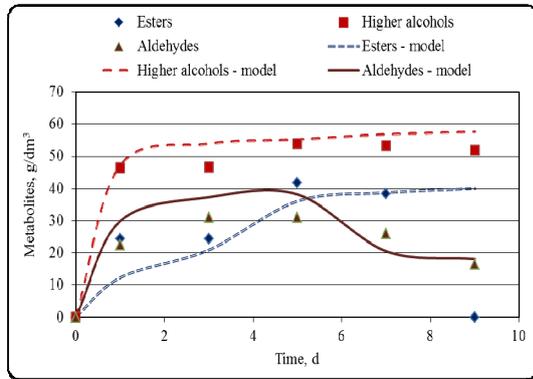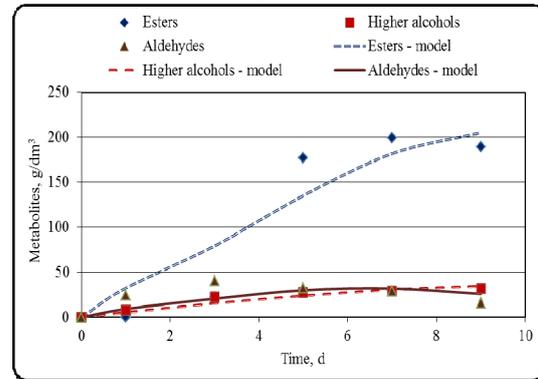

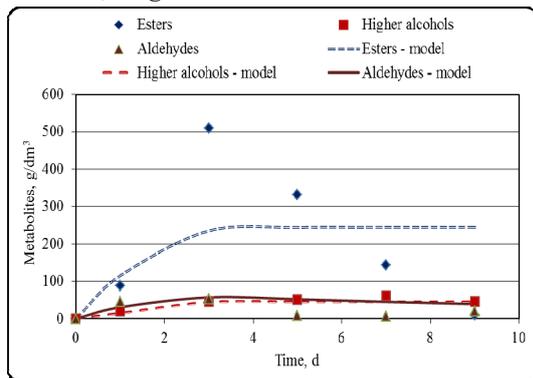a) free cells; original extract 9%
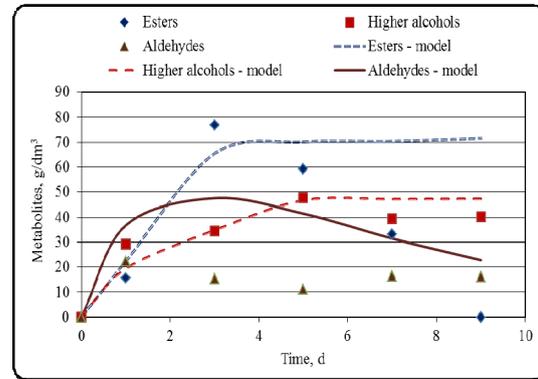
b) immobilized cells; original extract 9%
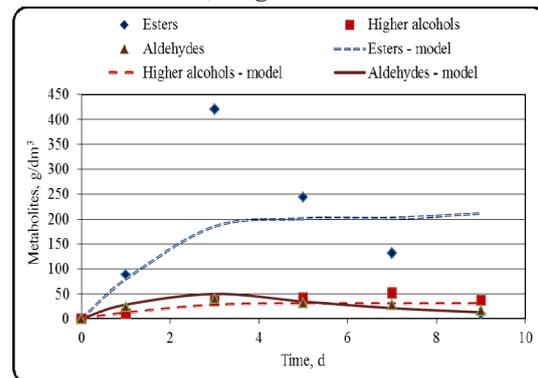

c) free cells; original extract 11%

d) immobilized cells; original extract 11%

e) free cells; original extract 13%

f) immobilized cells; original extract 13%

Figure 2 Experimental data and mathematical models for esters, higher alcohols and aldehydes during beer fermentation with free and immobilized cells of yeast strain Saccharomyces cerevisiae S-33

Higher alcohols
The immobilized cells produced more fusel alcohols than the free ones of yeast strain *S. pastorianus S-23*. The value of η_μ (Table 2) confirms the suggestion that rapid yeast growth of immobilized cells leads to enhanced anabolic production of amino acid precursors with concomitant synthesis of higher alcohols (Willaert and Nedovic, 2006). Decreased higher alcohol production by immobilized cells as opposed to free cell fermentation was shown in Figure 2. Mass (i.e. amino acids) transfer limitations results in decreased higher alcohol synthesis (Willaert and Nedovic, 2006). Table 2 shows that reduced synthesis of higher alcohols was observed for the majority of the studied variants with immobilized cells. The reduction varied between 8 and 68%. However, two of studied variants showed

enhanced synthesis of higher alcohols. It can be attributed to the diffusion resistances and the local changes in the diffusion coefficients of metabolites. The proposed mathematical model describes the experimental data with high accuracy and takes into account all the specificities of the fermentation process.

Aldehydes

There were significant differences in the aldehyde synthesis and reduction by immobilized and free cells. The free cells of both yeast strains showed distinct maximum of aldehydes, whereafter the aldehydes reduction started. On the other hand, the aldehyde peaks were not so distinct for the immobilized cells and the aldehydes concentration was relatively constant during fermentation. The explanation for this difference can be found in the kinetic parameters (Table 1 and Table 2). The specific rate of aldehydes synthesis of almost all immobilized cells of yeast strain *S. pastorianus* S-23 was higher than the one of free cells. It decreased with the increase of original wort extract. The specific rate of aldehydes synthesis of immobilized cells of yeast strain *S. cerevisiae* S-33 varied with different original wort extracts. The differences can be determined by the diffusion resistance, which are different in each case of fermentation.

Table 2

Efficiency coefficients for the description of fermentation with immobilized cells

Original extract	η_{μ} *	η_{q} *	η_{e}	η_{fa}	η_{a}	η_{ka}
			mg/(g.d)		-	
Saccharomyces pastorianus S-23						
9%	0.708	1.350	1.065	0.373	2.105	0.891
11%	2.564	2.724	1.239	1.641	1.562	3.500
13%	1.339	1.276	0.520	0.599	0.536	2.052
Saccharomyces cerevisiae S-33						
9%	1.248	0.914	1.305	0.923	0.665	1.333
11%	0.918	0.695	0.629	0.322	0.422	1.507
13%	0.559	0.879	0.827	1.201	1.173	1.776

* according to Parcunev et. al., 2012

The specific rate of aldehydes reduction depends on the current aldehydes concentration in beer and the biomass concentration in the working volume of the reactor. Table 2 shows that almost all specific rates of aldehydes reduction of immobilized cells were between 1.3 and 3.5 times higher than the ones of free cells. There was an exception with similar values of specific rates of aldehydes reduction between free and immobilized cells. It can be concluded that leads to the observed differences in this fermentation. Rapid reduction of aldehydes resulted in the retention of almost constant concentration of these compounds in beer produced with immobilized cells. At the end of fermentation the aldehyde concentration in all beers varied between 20 and 40 mg/dm^3.

The specific rate of aldehydes reduction can be increased by the increase of the temperature at the end of the fermentation process (maturation).

As it was previously mentioned, another group of compounds - vicinal diketones affects the beer flavor profile. Their synthesis is associated with yeast growth and their reduction has two stages - chemical and biochemical. The biochemical synthesis and reduction of vicinal diketones can be described with a similar model as equation (6). The chemical reduction depends on fermentation and maturation temperature, the mass transfer, the ethanol concentration in the beer, and etc. The modeling of synthesis and reduction of vicinal diketones will be the subject of further investigations.

The results of experimental and model data showed that the immobilization had no significant effect on the yeast metabolism. The flavor profile of beers produced with immobilized and free cells was comparable because the concentrations of the main groups of yeast by-products were similar. The observed differences in the synthesis and reduction of the main groups of metabolites had no effect on the beer quality. It can be suggested that the chosen carrier for immobilization was proper for implementation in beer technology because it did not affect the fermentation.

CONCLUSION

A mathematical model for the description of the synthesis and reduction of main flavor-active yeast by-products - esters, aldehydes and higher alcohols was developed and validated. The model described with high accuracy the beer fermentations with immobilized and free cells and confirmed the experimental data. Moreover, the results were in accordance to the previous study (Parcunev et al, 2012) and showed the impact of immobilization on the fermentation activity and metabolism of the yeast. The model did not take into account some biochemical and physicochemical processes during fermentation, but they are not essential for its accuracy.

ACKNOWLEDGEMENTS

LIST OF SYMBOLS

$X(t)$ – biomass concentration, g/dm^3; $P(t)$ – ethanol concentration, g/dm^3; $S(t)$ – substrate (extract) concentration, g/dm^3; μ - biomass specific growth rate, h^{-1}; μ_{max} – maximal biomass specific growth rate, h^{-1}; q_p – ethanol specific accumulation rate, g/(g.h); q_{pmax} – ethanol specific accumulation rate, g/(g.h); K_{SX} – Monod constant for the substrate, g/dm^3; K_{SP} – Monod constant for the product,

g/dm^3; FA – fusel (higher) alcohol concentration, g/dm^3; E – esters concentration, g/dm^3; A – aldehydes concentration, g/dm^3; Y$_{X/S}$ – yield coefficient biomass from glucose; Y$_{P/S}$ - yield coefficient ethanol from glucose; Y$_{FA}$ – yield coefficient for fusel alcohols, mg/(g.d); Y$_E$ – yield coefficient for esters, mg/(g.d); Y$_A$ – yield coefficient for aldehyds, mg/(g.d); k$_A$ – reduction coefficient for aldehydes, mg/(g.d); η – efficiency coefficient; ($_{μ,\ q,\ FA,\ E,\ Al,\ KAL}$ denotes the experimental parameters) E(r) – error between the experimental and model data; free – free cells fermentation; imm – immobilized cells fermentation;

REFERENCES

Branyik, T.; A. Vicente; P. Dostalek; and J.A. Teixeira. 2005 "Continuous beer fermentation using immobilized yeast cell bioreactor systems", *Biotechnol. Prog.*, 21, 653-663

Briggs, D.; C. Boulton; P. Brookes; and R. Stevens. 2004. *Brewing science and practice*, Woodhead publishing in food and science, CRC Press

Boulton C. and D. Quain 2001. "Brewing yeast and fermentation." *Blackwell Science*, ISBN 0-632-05475-1

Engan, S., 1981."Beer composition: volatile substances", In: Pollock, J.R.A (editor) Brewing Science,., *Academic Press*, vol. 2, p.98-105.

Dufour, J.-P. , Malcorps, Ph., Silcock, P., 2003 "Control of ester synthesis during brewery fermentation, In: Brewing yeast and fermentation performance", Smart, K. (Editor), *Blackwell Science*, 213-234

Handbook of brewing: Processes, Technology, Markets. 2009. Hans Michael Esslinger (ed.), ISBN 978-3-527-31674-8, *WILEY-VCH Verlag GmbH & Co. KGaA,* Weinheim

Kostov G.; S. Popova; V. Gochev; P. Kpoprinkova-Hristova; M. Angelov. 2012. "Modeling of batch alcohol fermentation with free and immobilized yeasts Saccharomyces cerevisiae 46 EVD." *Biotechnology and Biotechnological equipment*, ISSN 1310-2818, Vol. 26, 3, doi: 10.5504/bbeq.2012.0025

Kunze W. 2003. "Technology of brewing and malting, 3rd edition", ISBN 3-921690-49-8, *VLB-Berlin*

Marinov M. 2010. "Practice for analysis and control of alcohol beverages and ethanol", *Academic Publisher of UFT*, ISBN 987-954-24-0150-6, p. 196 (in bulgarian)

Meilgaard, M. 1975. "Flavour chemistry of beer. Part 2: Flavour and threshold of 239 aroma volatiles", *Technical Quarterly of the Master Brewers Association of the Americas,* 12, 151-168.

Mitev S.V.; S. B. Popova. 1995. "A model of yeast cultivation based on morphophysiological parameters." *J. Chemical and Biochemical Engineering Quarterly*, 3, Zagreb, 119-121.

Parcunev, I, V. Naydenova; G. Kostov; Y. Yanakiev, Zh. Popova; M Kaneva; I. Ignatov, 2012. "Modelling of alcoholic fermentation in brewing – some practical approaches". In: Troitzsch, K. G, Möhring., M. and Lotzmann, U. (Editors), Proceedings 26th European Conference on Modelling and Simulation, ISBN: 978-0-9564944-4-3, pp. 434-440

Popova S. 1997. "Parameter identification of a model of yeast cultivation process with neural network", Bioprocess and Biosystems Engineering, 16(4), 243-245, DOI: 10.1007/s004490050315

Ramirez W.F., Maciejowski, J. 2007. "Optimal beer fermentation" J. Inst. Brew. 113(3), 325–333

Rusell I., 2006, "Yeast", In: Priest F.G. and Stewart G.G (Editors) Handbook of brewing, *Taylor & Francis Group*, LLC, 281-333

Shen H.-Y., Moonjai N., Verstrepen K., Delvaux F., 2003. "Impact of attachment immobilization on yeast physiology and fermentation performance". *J. Am. Soc. Brew. Chem.*, 61(2), 79-87

Willaert R., 2007." The beer brewing process: wort production and beer fermentation". In: Y.H. Hui (Editor) Handbook of food products manufacturing, *John Wiley & Sons, Inc.*, Hoboken, New Jersey, 443-507

Willaert R., Nedovic, V. 2006. "Primary beer fermentation by immobilised yeast – a review on flavour formation and control strategies". *J Chem Technol Biotechnol*, 81, 1353–136

AUTHOR BIOGRAPHIES

GEORGI KOSTOV is associated professor at the department "Technology of wine and brewery" at University of Food Technologies, Plovdiv. He received his MSc in "Mechanical engineering" in 2007 and PhD on "Mechanical engineering in food and flavor industry. His research interests are in the area of bioreactors' construction, biotechnology, microbial population's investigation and modeling, hydrodynamics and mass transfer problems, fermentation kinetics.

SILVIA POPOVA. Associate Professor Dr. Silviya Popova received her MSc in mathematics from University of Sofia, Bulgaria (1977) and PhD on "New methods for automatization of videomicroscopy microbiological investigation" from the Bulgarian Academy of Sciences (2001). Her research interests are in modeling and identification of biotechnological processes, estimation and control of biotechnological processes, adaptive control, neural networks and image processing.

VESSELA NAYDENOVA is a PhD student at the department "Technology of wine and brewery" at University of Food Technologies, Plovdiv. Her research interests are in the area of beer fermentation with free and immobilized cells; yeast specification and fermentation activity. The PhD thesis is named "Possibilities for beer production with immobilized yeast cells"

VASIL ILIEV is a PhD student at the department "Technology of wine and brewery" at University of Food Technologies, Plovdiv. His research interests are in the area immobilized cells reactors modeling and application. The PhD thesis is named "Intensification of the processes in column bioreactor with immobilized cells"

MARIA KANEVA is assistant professor at the department "Technology of wine and brewery" at University of Food Technologies, Plovdiv. Her research interests are in the area on non-alcoholic beverages, herbal extracts for beverages, modeling of extraction processes.

STOYAN VASSILEV is a student at the department "Technology of wine and brewery". His bachelor degree thesis is on the subject of "Investigation on alcoholic fermentation for obtaining beer with free and immobilized top fermented yeast"

MARIANA BADOVA is a student at the department "Technology of wine and brewery" at University of Food Technologies, Plovdiv. Her bachelor degree thesis is on the subject of "Investigation on alcoholic fermentation for obtaining beer with free and immobilized bottom fermented yeast"