MODELING OF ALCOHOL FERMENTATION IN BREWING – INTEGRATED APPROACH FOR CONTROL OF CONTINUOUS ALCOHOL FERMENTATION

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KEYWORDS

Continuous beer fermentation, modeling, degree of fermentation, dilution rate, transitional mode

ABSTRACT

An integrated approach for the management of continuous beer fermentation with immobilized cells was developed. Experimental results for continuous fermentation were used for the development of dependencies for the calculation of real extract and ethanol at different degrees of fermentation and dilution rates. For this purpose, mathematical relationships describing the fermentation with high accuracy were used.

INTRODUCTION

Fermentation and maturation are the most time-consuming steps in the entire beer production process. In view of the highly competitive market, the potential time saving involved in immobilized cell technology (ICT) needs to be taken into account. ICT allows beer production to be accomplished in as little as 2-3 days (Branyik et al, 2005).

One of the ICT major advantages is the increased volumetric productivity due to the high volumetric cell densities in the reactor. Consequently, the fermentation can be performed in a smaller bioreactor (decreased capital costs) with shorter residence times (Willaert, 2007).

The first ICT process for beer production was developed in 1971. Yeast cells were immobilized in kieselguhr (which is widely used in the brewing industry as a filter aid), and a kieselguhr filter was employed as the bioreactor (called the "bio-brew bioreactor"). The serious problem of this system was the high amount of a-acetolactate in the green beer (Narziss and Hellich, 1972; Narziss, 1997). Nevertheless, over the last 30 years, immobilized cell technology (ICT) has been extensively examined and some designs have already reached commercial exploitation (Nedovic et al., 2005; Willaert, 2007). The main differences between the commercial systems are the carrier materials, the bioreactor type and the fermentation control (Van de Winkel et al., 1995; Andries et al., 1995; Andries et al., 1996; Leskosec-Cucalovic and Nedovic, 2005).

A major challenge for the successful application of ICT on an industrial scale is the control of the flavor profile during fermentation, as many parameters can have an influence on flavor formation (Willaert and Nedovic 2006). Therefore, the aim of this study is the development of dependences for the management of continuous alcoholic fermentation with immobilized bottom-fermenting yeast.

MATERIALS AND METHODS

Yeast strain and immobilization procedure

The fermentations were carried out with bottom-fermenting yeast strain *Saccharomyces pastorianus* Saflager S-23. The immobilization procedure was previously reported in (Parcunev et al., 2012).

Packed-bed reactor

The system used for the continuous beer fermentation is shown in Figure 1. It consisted of a column (6) having the following dimensions: total height = 1000 mm, working height = 800 mm and inside diameter = 30 mm. The fermentation temperature was maintained by means of the column water jacket and a water bath (4). The 1 mm thick grid (10) was placed inside the column. Thus, some space was formed between the column wall and the grid, which allowed the CO_2 formed during the fermentation to leave the packed bed without destroying it The immobilized cells were placed on a drainage bed (5) set at the bottom of the column. Sterile wort was charged into a tank (1), and the "green" beer was collected in another tank (9). The liquid level in the column was maintained by means of an overflow (7), and the pressure in the column could be controlled via a connection (8). The column was operated at a slight overpressure determined by the partial pressure of CO_2. When necessary, the pressure in the column was lowered using a small valve, thereby regulating the rate of CO_2 removal from the capsules. It could also be regulated through the clamping force of the plug in the top of the column. The liquid was fed into the column by a peristaltic pump (2) and pipes (3).

Starting and operating a packed bed reactor

The column was sterilized using disinfectants and washed with sterile water before operation. The drainage bed and the grid (10) previously sterilized in a boiling

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water bath were placed at the bottom of the column, Prior to inoculation of the reactor, the immobilized cells were placed in 400 cm^3 sterile wort in a sterile tank for 3-4 hours. It was necessary for the fermentation rapid start which resulted in reduction of the transitional mode time. The reactor was aseptically filled with the immobilized cells and the fermenting medium. Thus, a layer with a height of approximately 20 cm was formed. The height corresponded to 1/3 of the working height of the column after the drainage bed and the grid had been placed. The immobilized cells mass was 48 g, which was 4 times as high as the optimal mass determined for batch beer fermentation. The fermenting wort amount in the column was adjusted to 400 cm^3. The fermentation temperature was maintained in the range of 16 °C. The column was supplied with wort from tank (1) by means of a peristaltic pump at a flow rate that ensured the appropriate dilution rate. The dilution rate was determined on the basis of the volume of the fermenting wort in the column: 400 cm^3. The "green" beer leaked through the outflow and was collected in the "green" beer tank (10). The residence time in the column was inversely proportional to the dilution rate.

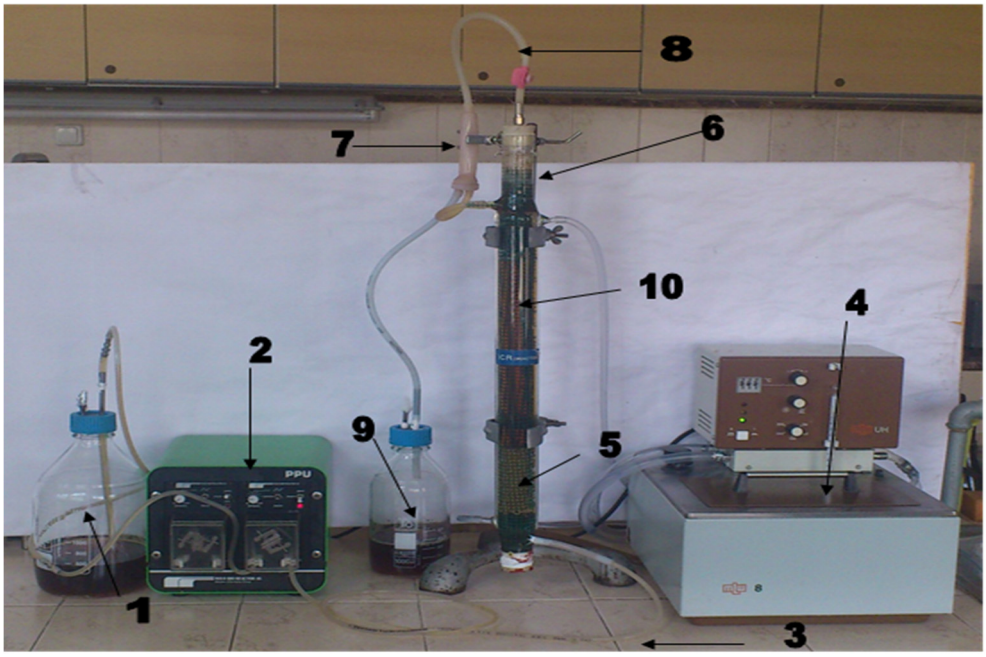


Figure 1. System for continuous beer fermentation with immobilized cells
1: wort tank; 2: peristaltic pump; 3: pipes; 4: water bath; 5: packed bed; 6: column; 7: overflow; 8:pressure control connection; 9: "green" beer tank; 10: grid

Analytical methods

The characterization of wort, green beer and beer (original extract, degree of fermentation, extract, and alcohol) was conducted according to the current methods recommended by the European Brewery Convention (Analytica-EBC, 2004).

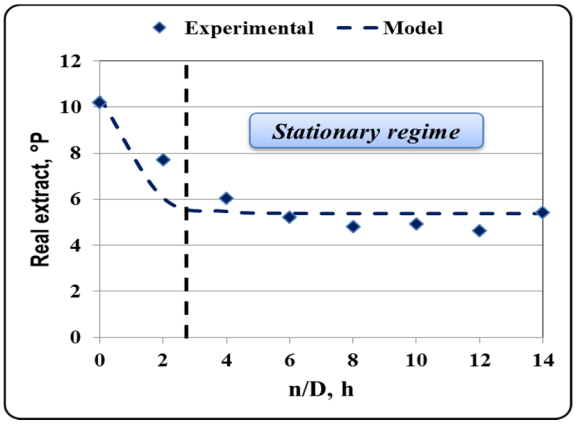
RESULTS AND DISCUSSION

Development of models for continuous fermentation management

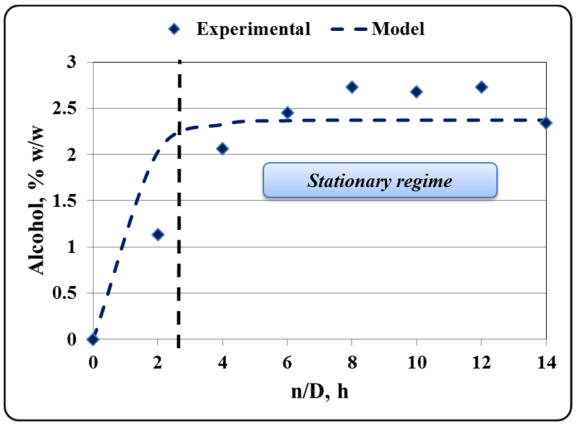
In our previous studies (Parcunev et al., 2012; Vassilev, 2013; Naydenova, 2014; Naydenova et al, 2014), it was found that for batch beer fermentation there was a zone of optimum values of some of the operational parameters, which resulted in minimal fermentation time. The optimal batch fermentation parameters were as follows: original extract: 9.72 °P; immobilized cell mass: 12.71 g; main fermentation temperature: 16 °C; maturation temperature: 20 °C (Naydenova, 2014; Naydenova et al, 2014). The batch fermentation could be transferred into a continuous mode and then the dilution rate had to be optimized.

Therefore, three continuous fermentations were made with the following dilution rates: 0.0825 h^{-1}; 0.163 h^{-1} and 0.25 h^{-1}. These dilution rates corresponded to a main fermentation time of 12, 6 and 4 h, respectively. The fermentation times were much shorter than the optimal main fermentation time of 72 h during batch fermentation. Meanwhile, the results showed that the beers produced in batch and continuous modes were within the commercial product range (Naydenova, 2014). Figure 2 presents the data on the dynamics of one of the continuous fermentations. The results showed that the continuous fermentation system studied worked in two modes: transitional and stationary. The transitional mode varied between 60 and 72 h, which corresponded to the optimal main fermentation time during the batch fermentation. If the transitional mode was considered dimensionless, it was within the range of 3/D-8/D, which corresponded to the data cited in (Malek and Fenel, 1966). This mode was necessary for the immobilized

cells to reach a certain physiological state. Therefore, for industrial scale-up, it is necessary to carry out batch main fermentation, which will replace the transitional mode. During the stationary mode, the continuous fermentation system worked steadily with reproducible characteristics. The trends for the other two continuous fermentations were similar (see Figure 3). In summary, the apparent degree of fermentation in the stationary mode varied between 40% and 64%, and decreased with the increase in dilution rate. The prolonged residence time led to an increase in extract consumption and ethanol production.



a) extract



b) alcohol

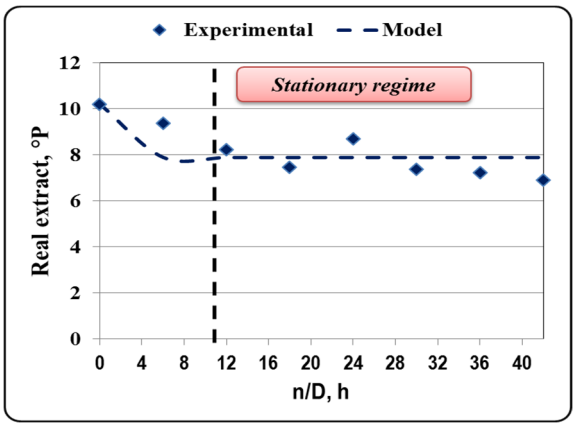
Figure 2. The dynamics of extract consumption and ethanol production at dilution rate D=0.0825 h^{-1}

A simple exponential dependence was selected for the description of the fermentation dynamics in the continuous mode:

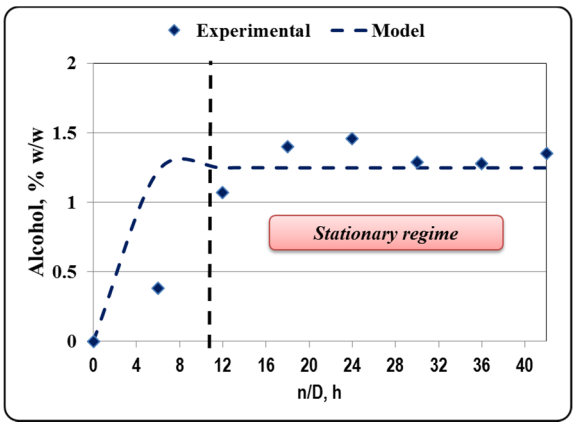
$$y = a + be^{-x} \quad (1)$$

The non-dimensional fermentation time - n/D was chosen as a parameter in the equation . Thus, calculations of the operational parameters could be made more easily for each fermentation stage.

This model type was chosen because of the experimental curve form. In the transitional mode, the extract decreased exponentially to a certain fixed value determined by the degree of fermentation. On the other hand, the alcohol increased exponentially. It is known that the simplest way of describing the fermentation process is by using a simple exponential equation (Malek and Fenel, 1966).



a) extract



b) alcohol

Figure 3. The dynamics of extract consumption and ethanol production at dilution rate D=0.25 h^{-1}

The data on the model constants are presented in Table 1. They were determined from the equations for real extract and alcohol. With regard to real extract, the sum of coefficients **a** and **b** was practically equal to the original wort extract. The value of coefficient **a** was similar to the value of the average real extract in the stationary mode and coefficient **b** coincided with the amount of fermented extract in the stationary mode of the system. With regard to alcohol, the value of coefficient **a** was equal to the average alcohol concentration in stationary mode. Interestingly, the absolute value of coefficient **b** was also similar to the average alcohol concentration in the stationary mode.

The relationship between the constants in equation (1) is represented by the fermentation degree, which is well known in brewing. The degree of fermentation represents the percentage of extract, which has been transformed to ethanol (Kunze, 2003). It can be concluded that there was a relationship between the values of coefficients **a** and **b**. In brewing, it is determined by the Balling equation which was studied in our previous publication (Parcunev et al., 2012):

$$\underset{extract}{2,0665\ g} \rightarrow \underset{alcohol}{1,0000\ g} + \underset{CO_2}{0,9565\ g} + \underset{biomass}{0,11\ g} \quad (2a)$$

which could be written in the following way:

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

By means of the Balling equation and the theoretical ethanol yield from glucose the following two equations summarizing the relationship between coefficients **a** and **b** for real extract and ethanol could be written:

$$RE = (1 - f)OE + fOEe^{-n/D} \quad (3)$$

where: RE: real beer extract, °P; OE: original wort extract, °P; f: degree of fermentation; D: dilution rate, h^{-1}; n: constant which takes values 1,2,…n.

$$Alc = 0.51fOE - 0.51fOEe^{-n/D} = 0.51fOE(1 - e^{-n/D}) \quad (4)$$

where: OE: original wort extract, °P; f: degree of fermentation; D: dilution rate, h^{-1}; n: constant which takes values 1,2,…n.; 0.51: theoretical ethanol yield from glucose

Table 1

Values of the coefficients in equation 1

Dilution rate D, h^{-1}	Real extract			Alcohol		
	a	b	R^2	a	b	R^2
0.0825	5.39	5.039	0.835	2.37	-2.504	0.746
0.163	6.336	3.903	0.764	2.25	-2.025	0.744
0.25	7.894	2.319	0.691	1.25	-1.25	0.644

The main parameters in equations (3) and (4) were the degree of fermentation and constant n. The degree of fermentation depended on the type of beer produced. The constant n took positive integers and did not depend on the selected fermentation mode. The third parameter in the equations (3) and (4) was dilution rate. It defined the degree of fermentation but on the other hand, it depended on this parameter. Therefore, the management of a continuous fermentation system can be performed at a set value of D and defined degree of fermentation and original wort extract.

Comparative study of different modes of continuous beer fermentation

Equations (3) and (4) were used for a simulation study on the influence of the degree of fermentation and dilution rate on several modes of continuous beer fermentation.

In the transitional mode, the degree of fermentation constantly grew to the preset one. The dilution rate affected the transitional regime duration at the same degree of fermentation (Figure 4 and Figure 5). The transitional mode duration varied between 6 at D = 0.1 h^{-1} (Figure 4) and 10 at D=0.2 h^{-1} (Figure 5). According to (Malek and Fenel, 1966), the transitional mode had to continue for 3/D. It was not confirmed by the results on the simulated fermentations, which was probably due to the influence of the degree of fermentation. The time when the transition to stationary mode occurred has been represented by vertical dash lines (Figure 4 and Figure 5). The effect of the dilution rate on the transitional mode duration has been summarized in Figure 6. The increase in the dilution rate resulted in a prolonged non-stationary phase. The results indicated that equations (3) and (4) could be used for the selection of modes for the production of low-alcohol and non-alcoholic beers. The modes appropriate for the production of these beer types had dilution rates over 0.35 h^{-1} and were characterized by a relatively long non-stationary phase. The beers produced during the transitional mode would be similar to the beers produced in the stationary mode.

To prove the hypothesis that the degree of fermentation affected the transitional regime duration, three fermentations were simulated at dilution rate 0.1 h^{-1} and degree of fermentation 60%, 70% and 75%, respectively. The results are shown in Figure 7 to Figure 9.

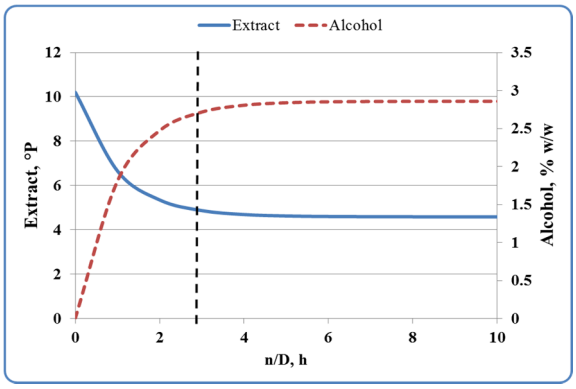


Figure 4. Fermentation dynamics at dilution rate 0.1 h^{-1} and degree of fermentation 55%

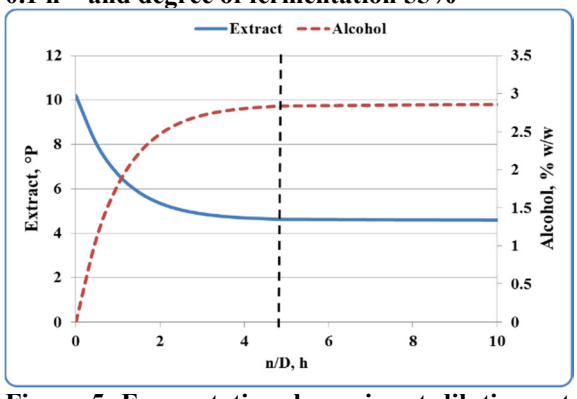


Figure 5. Fermentation dynamics at dilution rate 0.2 h-1 and degree of fermentation 55%

All the data on the effect of the degree of fermentation on the transitional mode duration have been summarized in Figure 10. The results showed that the non-stationary phase duration depended on the selected degree of fermentation. The increase in the degree of fermentation led to an increase in the transitional mode duration and the production of beer of non-permanent quality. Therefore, the main prerequisite for the reduction of the transitional mode time and the stable work of the system

in the stationary phase should be the decrease in the degree of fermentation and the dilution rate. Moreover, the changes in these two parameters had to be connected with the beer flavor profile and amount of the extract which had to ferment during the beer maturation.

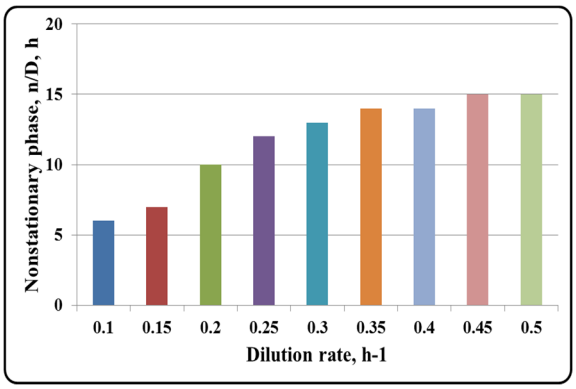


Figure 6. Effect of the dilution rate on the non-stationary phase duration at degree of fermentation 60%

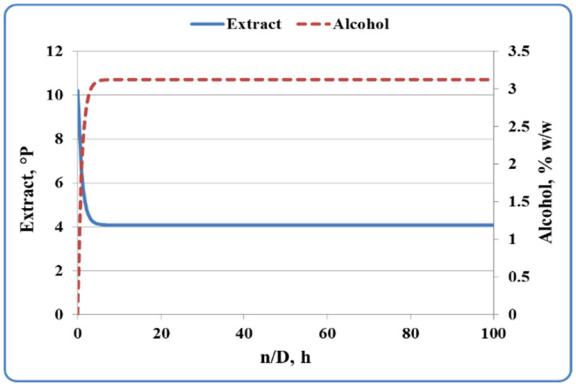


Figure 7. Fermentation dynamics at dilution rate 0.1 h^{-1} and degree of fermentation 60%

The data in Figure 7 to Figure 9 show that the influence of the degree of fermentation on the continuous beer fermentation was stronger than the effect of the dilution rate. It can be hypothesized that the increase in the original wort extract, especially in high-gravity beer production, will lead to an increase in the effect of the degree of fermentation. Therefore, it is necessary to seek a compromise between the choice of the fermentation mode and the type of the beer produced.

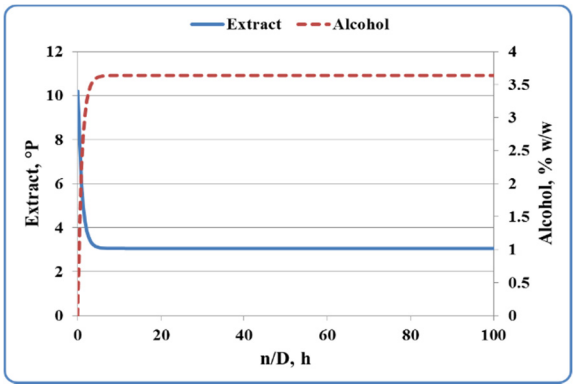


Figure 8. Fermentation dynamics at dilution rate 0.1 h^{-1} and degree of fermentation 70%

CONCLUSION

A simplified method for the calculation of the main parameters of continuous beer fermentation with immobilized yeasts was developed. The method was based on the data for continuous fermentation in a packed bed column bioreactor. The selected exponential equations allowed the description of the transitional and stationary mode of the system. Regression analysis was used for the development of dependencies which could be used for the calculations of the real extract and alcohol at various degrees of fermentation and dilution rates. The equations developed were used for the determination of the effect of the degree of fermentation and dilution rate on the non-stationary phase duration, and for the efficient management of the continuous fermentation system.

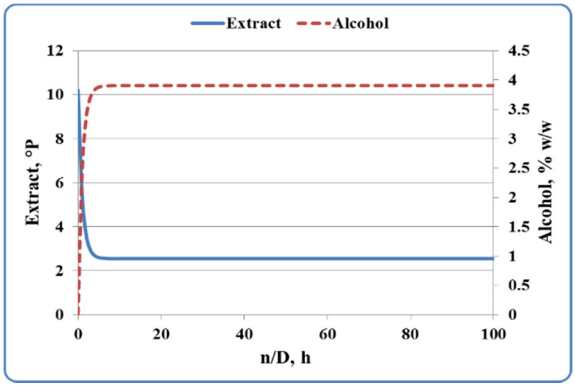


Figure 9. Fermentation dynamics at dilution rate 0.1 h^{-1} and degree of fermentation 75%

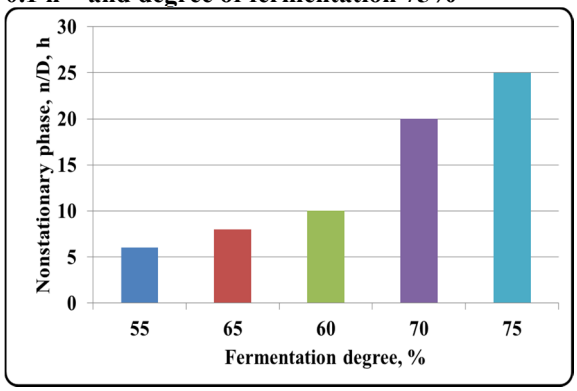


Figure 10. The effect of the degree of fermentation on the non-stationary phase duration

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