Possibilities for the biological acidification of mash in the production of wort: Kinetics of lactic acid production in a free cell culture of *Lactobacillus delbrueckii* ssp. *bulgaricus* M3

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Possibilities for the biological acidification of mash in the production of wort: Kinetics of lactic acid production in a free cell culture of Lactobacillus delbrueckii ssp. bulgaricus M3. *The kinetic relation of the accumulation of lactic acid, diacetyl and biomass during the cultivation of Lactobacillus delbrueckii ssp. bulgaricus M3 was examined. The logical equation and the model of Luedeking-Piret were used for the modeling of the kinetics. The model parameters were identified and the specific features of the utilization of carbohydrates and lactic acid accumulation were shown.*

Key words: *brewing, mashing, acidification, lactic acid production, lactic acid bacteria, corn extract*

INTRODUCTION

The process of saccharification of malt mash is essential for obtaining wort. During this process the high molecular weight substances of malt and the unmalted raw materials are converted into soluble form and pass into the wort. The mashing process depends on various factors like pH, temperature and malt mash concentration. pH plays an important role in the saccharification of malt mash because each enzyme involved in the process is characterized by a certain pH optimum. The amylase complex that digests starch is characterized by a pH optimum in the area of 5.3 - 5.6. At pH values in the range of 5.3 to 5.4 an increase in the yield of the extract, improvement of the filtration as well as improvement of the color characteristics of the obtained wort is observed [4, 5].

Biological acidification of malt mash using lactic acid concentrates is a widely used technique for pH adjustment in beer production. The lactic acid concentrates are obtained by the cultivation of lactic acid bacteria strains of the species: *L. delbrueckii* ssp. *delbrueckii*, *L. delbrueckii* ssp. *lactis, L. fermentum, L. amylolyticus*, *L. amylovorus* and others [7]. The advantages of this method of pH adjustment include higher extract yield, higher rate of filtration of the slurry, better fermentation, more harmonic taste of the obtained beer, improvement of the foaming and the colloidal and flavor stability. An important requirement in the selection of lactobacilli strains is the production of maximum amounts of lactic acid and relatively low concentrations of diacetyl [4, 7].

It is of major importance to have knowledge on the kinetics of the lactic acid fermentation process. Mathematical modeling is a fundamental method for studying the kinetics of the cultivation process [1, 2, 8].

The aim of the present work was to study the kinetics of the process of lactic acid production with free cells of *Lactobacillus delbrueckii* ssp. *bulgaricus* M3 for the biological acidification of mash in the preparation of wort.

MATERIALS AND METHODS

Microorganisms

The strain used in the present paper is *Lactobacillus delbrueckii* ssp. *bulgaricus* M3 from the collection of the Department of “Microbiology” at the University of Food Technologies, Plovdiv.

Media and culture conditions

1. MRS-agar (Scharlau)

2. Fermentation broth. Sweet wort with initial extract concentration - 20 °P. The sweet wort was diluted in a ratio of 1:1. The medium was supplemented with 1% corn extract. The fermentation medium was sterilized at 121 °C for 25 min.

3. Cultivation conditions. The cultivation was carried out in a laboratory bioreactor with a working volume of 1.5 dm^3. The bioreactor was equipped with a control unit

"Sartorius A2" that included control devices for the stirring rate, the temperature, the pH and other parameters.

Determination of the amount of lactic acid

For the determination of the acid-forming ability of the lactic acid bacteria, the titratable acidity (expressed as Toerner degree, °T) was determined by titration of 10cm^3 sample with 0.1N NaOH until the appearance of a pale pink colour using phenolphthalein. 1°T equals 0.009 g lactic acid [12, 13].

Determination of the concentration of viable lactobacilli cells

Appropriate tenfold dillusions of the samples in saline solution were prepared. They were spread onto Petri dishes with MRS-agar medium and incubated for 72 hours at 37°C until single colonies were formed [12, 13].

Results are shown as the average values from three independent experiments. Microsoft Excel 2007 was used for data analysis.

Determination of total vicinal diketones

Vicinal diketones (VDK) were determined spectrophotometrically [9]. 100 cm^3 of each sample were subjected to steam distillation. The rate of the distillation was maintained in the range of 3 cm^3 of distillate per minute. It was necessary to collect 25 cm^3 of distillate. To 10 cm^3 of distillate were added 0.5 cm^3 of o-phenylenediamine solution (final concentration - 10 g/dm^3. The solution was prepared by dissolving o-phenylenediamine in 4M HCl). A blank sample containing distilled water and o-phenylenediamine solution and a standard sample containing 9.9 cm^3 of distilled water, 0.5 cm^3 of the o-phenylenediamine solution and 0.1 cm^3 of standard solution of diacetyl (concentration 250 mg/dm^3) were prepared as well. After loading the samples remained in the dark for 30 min, and then 2 cm^3 of 4M HCl were added to each sample and the absorbance at wavelength λ = 335 nm was measured. The diacetyl concentration was calculated according to the equation:

$$D = \frac{A_{335} - A_{np}}{A_{CT} - A_{np}}.0,625,\ mg / dm^3 \qquad (1)$$

Determination of reducing sugars (PAHBAH method)

The samples were decolorized with activated carbon and deproteinated with lead acetate [3]. They were then diluted with distilled water until discolorisation. 5µl of the diluted sample were mixed in a test tube with 1 cm^3 of a reagent composed of solution A and solution B. The two solutions were mixed immediately prior to use. The tubes were boiled in a boiling water bath for 6 minutes, cooled and the absorbance was measured at wavelength λ = 410 nm [6].

Identification of the model parameters

The model of the logistic equation (model of Verhulst) and the equation of Luedeking-Piret were used for the description of the kinetic parameters. The kinetic parameters were determined in analogy to [1]:

$$\psi = 1 - \frac{X_t}{X_{t+\Delta t}} = 1 - \left(1 - \frac{X}{X_K}\right)\exp(-\mu_m \Delta t) \qquad (2)$$

wherein: ψ - relative change of the microbial concentration; Δt - time interval of the change of the microbial concentration from X_t to $X_{t+\Delta t}$.

The specific rates of accumulation of the product in the equation of Luedeking-Piret (3) were determined by the method of the least squares [14].

RESULTS AND DISCUSSION

The dynamics of lactic acid production with free cells of *Lactobacillus delbrueckii* ssp. *bulgaricus* M3 is shown on Fig. 1. The process was conducted in a laboratory bioreactor with mechanical stirring. The data demonstrated great amount of reducing sugars in the end of the fermentation. The lag phase was about 6 hours and the culture entered the

stationary growth phase about the 36th hour. The concentration of active lactobacilli cells reached $2.5x10^{15}$cfu/cm^3. In the period of active fermentation around 12.42 g/dm^3 lactic acid were accumulated. During fermentation, the pH decreased from 5.5 to 4-4.2.

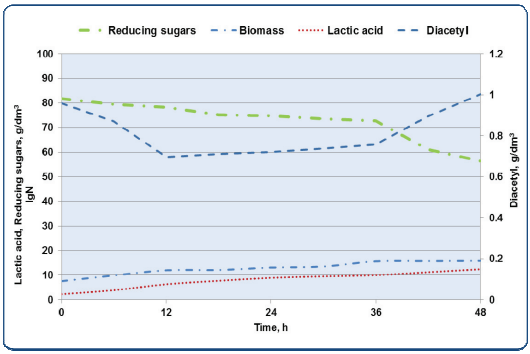


Fig. 1. Dynamics of lactic acid production during cultivation of *Lactobacillus delbrueckii* ssp. *bulgaricus* M3 in sweet wort in a laboratory bioreactor

An important prerequisite for the biological acidification of mash is the minimum production of diacetyl by lactic acid bacteria. By the 12th hour the cells actively produced diacetyl and its concentration reached 0.67 mg/dm^3. From the 12th to the 36th hour the rate of diacetyl production was relatively constant with slight variations in the diacetyl concentration. After the 36th hour there was an increase in the concentration of diacetyl and at the end of the process it was 1.002 mg/dm^3.

A number of mathematical models are used to describe the kinetics of lactic acid production. In most cases, the kinetic parameters in the models don't have clear biological meaning and they may be difficult to interpret from biological point of view [1, 2, 8].

In the present work the model of the logistic curve (the equation of Verhulst), combined with the equation of Luedeking-Piret were used to describe the kinetic parameters. After identification of the parameters a system of equations was obtained (5). The advantages of this model were the clear biological meaning of the parameters and the fact that they can quickly and easily be analyzed. The comparison of the experimental data with the model data is shown on Fig. 2. (The model parameters are shown below the figure.)

The mathematical model reflects experimental data with very high accuracy. The identified error (e = 0.363) is sufficiently small leading to a high correlation coefficient R^2 = 92.32%. The culture developed with a relatively high specific growth rate. The growth of the cells was not influenced by the culture conditions. This is indicated by the low value of the coefficient of internal population competition $\beta = 0.0089$ dm^3/(g.h).

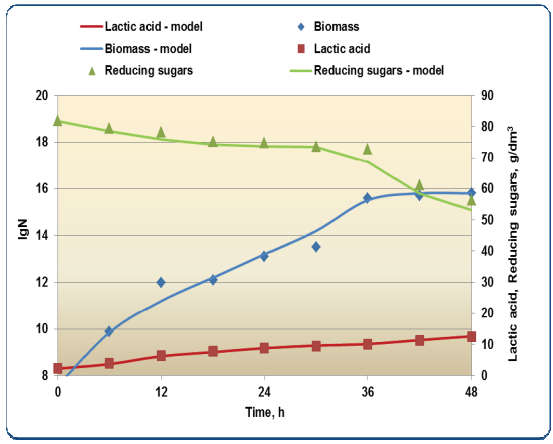
The accumulation of lactic acid was carried out by the cells that had already entered the stationary growth phase. The constant K = 1.651 g / (CFU.h) was 27 times higher than that of the member indicating the growth of the biomass - q_{p0} = 0.061 g/(CFU.h). This data corresponded to the results of our previous studies [2, 10, 11].

The higher value of the coefficient $\delta = 1.436$ compared with $\gamma = 0.0142$ showed that the basic amounts of carbohydrates were absorbed by cells in the stationary growth phase. This was due to the fact that this constant includes a factor involved in maintaining of the biological status of the lactobacilli population m_s. In addition to that the second member was directly related to the production of lactic acid, which again confirmed that stationary phase cells produce lactic acid. The growing population absorbed relatively

small amounts of carbohydrates, mainly to provide the necessary energy and building material.

$$\left|\begin{array}{l}\dfrac{dX}{d\tau}=\left[\mu_m-\beta X\right]X\\ \dfrac{dP}{d\tau}=KX+q_{p0}\dfrac{dX}{d\tau}\\ -\dfrac{dS}{d\tau}=\gamma\dfrac{dX}{d\tau}+\delta X\end{array}\right| \Rightarrow \left|\begin{array}{l} X=\dfrac{X_H e^{\mu_{\max}(t-tl_{ag})}}{1-\dfrac{X_H}{X_{KP}}\left(1-e^{-\mu_{\max}(t-tl_{ag})}\right)}\\ P=P_0+K\left(X-X_H\right)+q_{p0}\left(\dfrac{X_K}{\mu_m}\right)\ln\left(1-\dfrac{X_H}{X_K}\left(1-e^{-\mu_m\tau}\right)\right)\\ S=S_0-\gamma X_0\left[\dfrac{e^{\mu_m\tau}}{1-\dfrac{X_0}{X_m}\left(1-e^{\mu_m\tau}\right)}-1\right]-\delta\dfrac{X_m}{\mu_m}\ln\left[1-\dfrac{X_0}{X_m}\left(1-e^{\mu_m\tau}\right)\right]\end{array}\right| \qquad (3)$$

wherein: μ_{max} - maximum specific growth rate, h^{-1}; β - coefficient of internal population competition, $dm^3/(g.h)$; K - constant of product accumulation from biomass in the stationary growth phase, g/(CFU.h); q_{p0} - constant of product accumulation from the growing biomass, g/(CFU.h); $\gamma = 1/Y'_{X/S} + K/Y'_{P/S}$ - constant of utilization of the substrate per unit biomass in the stationary growth phase, g/(CFU.h); $\delta = q_{P0}/Y'_{P/S} + m_s$ - constant of absorption of the substrate by the growing biomass and substrate for the maintenance of the culture, g/(CFU.h).



μ_{max} = 0.141 h^{-1}; β = 0.0089 $dm^3/(g.h)$; K = 1.651; q_{p0} = 0.061; δ = 1.436; γ = 0.0142; e = 0.363

Fig. 2. Comparison of the dynamics of fermentation in culturing *Lactobacillus delbrueckii* ssp. *bulgaricus* M3 applying the model of equation 3

It is interesting to note that the specific rates of diacetyl synthesis and reduction in the medium were relatively close. Unfortunately the used model did not allow looking for kinetic relations of the synthesis and the reduction of vicinal diketones. This was due to the fact that the reduction has a chemical moiety which cannot be assessed by a model with biological constants.

CONCLUSION

The kinetics of the batch processes for obtaining lactic acid with free cells of the strain *Lactobacillus delbrueckii* ssp. *bulgaricus* M3 during cultivation in fresh wort was studied and modeled. To model the kinetics of the process the logistic curve and the

model of Luedeking-Piret were used. This system of equations has a clear biological meaning. It was confirmed that the cells in the stationary phase of growth produced the major amount of lactic acid. Most of the carbohydrates were also metabolized by the cells in the stationary growth phase. The model did not allow looking for kinetic relations of the synthesis and the reduction of diacetyl because of the presence of purely chemical processes involved in the reduction of this compound.

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Acknowledgements:

The authors express their gratitude to the brewery “Kamenitza” Ltd, Plovdiv for the generous supply of wort for the conduction of the cultivation process.

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This paper has been reviewed