THE DETERMINATION OF KINETIC CONSTANTS AND PREDICTION OF THE MATHEMATICAL MODEL FOR PERIODIC FERMENTATION IN INDUSTRIAL CONDITIONS

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Abstract

Our research aims to study the fermentation process with free brewer's yeast, Saccharomyces carlsbergensis, under industrial conditions. The study of the process will focus on determining kinetic constants, such as the maximum specific growth rate $\mu_{\max}$ *and the half-saturation constant* K_s*, through the Office package and MATLAB, because it is necessary at first to understand the growth kinetics of microorganisms. We know that the kinetics of the growth varies not only from the type of the microorganism but also from the type of the process, such as periodic or continuous, which affects the fermentation conditions. The knowledge of these constants is necessary for a further step to move to a pilot program, where the fermentation process will be carried out with free and/or immobilized yeast in periodic and continuous conditions. From the calculation of these quantities, we can predict the course of the fermentation process or the maturation of the beer in industrial conditions. During the estimation of these constants, the fermentation process progressed normally across all tanks, as evidenced by the substrate consumption observed over the course of the fermentation period. During the evaluation of the kinetic constants, temperature was found to have a considerable influence. Choosing an appropriate mathematical model is complex. By looking at the features of each theoretical model and comparing them with the experimental results, we can say that the Teissier model fits the data better.*

Key words*: kinetic constants, fermentation, periodic process, mathematical models.*

Përmbledhje

Qëllimi i këtij kërkimi është të studiojë procesin e fermentimit me maja birre të lirë, Saccharomyces carlsbergensis, të kryer në kushte industriale. Studimi i procesit do të përqendrohet në përcaktimin e konstantave kinetike, si shpejtësia maksimale e rritjes së majasë dhe konstantes së gjysëm ngopjes, përmes paketës Office dhe programit MATLAB, pasi është e domosdoshme fillimisht të kuptohet kinetika e rritjes së mikroorganizmave. Duke ditur që kinetika e rritjes ndryshon jo vetëm sipas llojit të mikroorganizmit, por edhe sipas llojit të procesit, si për shembull periodik apo i vazhduar, çka ndikon drejtpërdrejt në kushtet e fermentimit. Njohja e këtyre konstantave është thelbësore për të hedhur hapin e mëtejshëm drejt një impianti pilot, ku procesi i fermentimit do të zhvillohet me maja të lirë dhe / ose të imobilizuara, në kushte periodike dhe të vazhduara. Nga llogaritja e këtyre konstantave, mund të parashikohet ecuria e procesit të fermentimit apo e maturimit të birrës në kushte industriale. Gjatë vlerësimit të konstantave u vu re se në përgjithësi, ecuria e procesit të fermentimit ka qënë e njëjtë në të gjitha tanket. Gjatë vlerësimit të konstantave kinetike u konstatua se temperatura kishte ndikim të ndjeshëm. Parashikimet e modeleve matematikore teorike dhe eksperimentale e bëjnë të vështirë përzgjedhjen e modelit më të përshtatshëm. Duke analizuar karakteristikat e secilit model teorik dhe duke i krahasuar me rezultatet eksperimentale, mund të thuhet se modeli i Teissier përputhet më mirë me të dhënat eksperimentale.

Fjalë kyçe: *konstantet kinetike, fermentim, process periodic, modelet matematike.*

Introduction

Fermentation is one of the oldest known biotechnological processes, and due to its numerous health benefits, it has been the subject of continuous study. (Doran *et al.*, 1997) Over time, scientific research has significantly expanded our understanding of this process, providing detailed insights into its role in meeting human biological needs (Bailey *et al.*, 1986), enhancing the nutritional properties of food products, and promoting sustainable practices for environmental preservation.(Shanina *et al.*, 2023)

The fermentation process significantly impacts human health, which continues to drive ongoing studies and research in this field. (Stewart *et al.*, 1986) Due to the broad benefits associated with the development of fermentation in various domains, such as the maximisation of biotransformations of food waste and the application of innovative techniques like precision fermentation, this process has been increasingly adapted for the production of proteins, carbohydrates, and other essential compounds. (Shanina *et al.*, 2023)

Given that the future of industrial fermentation is increasingly oriented toward continuous processing, the integration of automation systems is becoming essential. This transition reduces operational errors and significantly lowers production costs for both industrial and food-grade bioproducts. Within the framework of Industry 4.0, automated control systems enable real-time monitoring and dynamic adjustment of critical fermentation parameters—such as temperature, pH, dissolved oxygen, and nutrient flow—ensuring optimal yields of high-quality, sterile products. Moreover, such systems contribute by minimising energy and water usage, as well as reducing reliance on manual labour.

With the development of technology, there is a need to study it first in pilot conditions as a continuous process and then in industrial conditions (Di Serio *et al*., 2001). In order to follow the progress of the research, we must study the kinetic constants as rigorously as possible and then build the mathematical models that fit the fermentation process of beer with free yeast in industrial conditions (Terkida *et al*., 2017). The study aims to determine kinetic constants and build mathematical models of the beer fermentation process in industrial conditions with free yeast *Saccharomyces carlsbergensis* using different calculation methods. (Arellano – Plaza *et al.*, 2007) The quantities we calculate help us continue the study of the pilot plant with free and immobilized yeast continuously. (Terkida *et al*., 2017).

We monitored parameters such as the substrate, temperature, and time during the development.

Materials and methods

We have considered the progress of the industrial fermentation process in five tanks, which are almost under the same conditions. We have used beer wort, with an initial extract of 10 °Brix, inoculated with free *Saccharomyces carlsbergensis* yeast with a cell density of 8%, where the experiment was developed in the "Stefan & Co" brewery in Tirana. Glucose (substrate) was measured in different periods until a constant value was obtained. The periodic fermentation process was carried out in 1L bioreactor. In addition to monitoring the substrate, the fermentation temperature was also monitored, which varied from 10 °C to 14 °C. (Manaj *et al.*, 2018)

Kinetic parameters evaluation

Monod equation is the equation that expresses the specific rate from the concentration of the limiting substrate:

$$\mu = \mu_{\max} \frac{s}{K_s + s} \quad (1)$$

There are several types of linearisation, but we have used the Lineweaver-Burk linearisation of the above equation (Luljeta Pinguli *et al*., 2022). The kinetic parameters were calculated graphically based on the data from the periodic fermentation process carried out under industrial conditions (Nanba *et al*., 1987). The dependence is approximated by a straight line, where the equation is Formed, where the kinetic constants are calculated from the intersections with the respective axes:

$$\frac{1}{\mu} = \frac{K_s}{\mu_{\max}} \cdot \frac{1}{s} + \frac{1}{\mu_{\max}} \quad (2)$$

The obtained results will be given in the following sections.

Mathematical modeling

There are several mathematical models of microbial growth. However, we have used only models that do not influence the inhibition of the substrate or the product from the amount of biomass produced, the weight of the substrate, or even the temperature (Kostov *et al*., 2012). Models are listed below:

- The Monod model

One of the most used kinetic models is the Monod model studied by Bailey and Ollis in 1986:

$$\mu = \mu_{\max} \cdot \frac{s}{s + K_s} \quad (3)$$

μ - is the specific growth rate (h^{-1}); s - is the substrate concentration (gr/l); K_s – is the Monod constant; μ_{max} – is the maximum growth rate.

The kinetic parameter μ_{max} was calculated using a linear expression from the slope of the graph, showing the dependence of the inverse of the specific growth rate and the substrate. The value of K_s indicates the substrate concentration when μ is half of μ_{max}.

- A modified model of Monod

The modified model is another model with a slight modification of the Monod model:

$$\mu = \mu_{\max} \cdot \frac{s}{K_s \cdot s + s} \quad (4)$$

In this model, the concentration of the substrate is significant, so the kinetics are directly affected by the values of the substrate.

- The Teisser model

The Teisser model expresses the growth kinetics by relating μ to s exponentially. This model is taken from the Monod model and is given by the following equation:

$$\mu = \mu_{\max} \cdot \left(1 - \exp\left(-\frac{s}{K_s}\right)\right) \quad (5)$$

Results and discussion

The dependence of the substrate on time

A perfect way to track the progress of the fermentation process is to illustrate the dependence of the substrate on time. During the development of the fermentation process in the five tanks, we observed a decrease in the amount of substrate over time, which is where the fermentation process typically occurs, and a reduction in the amount was anticipated. In all the tanks, fermentation occurs almost identically.

Comparing them, the last tank (11M) performed better than the others due to the gradual increase in temperature throughout the fermentation process. Tank 12M displays a more rapid consumption of sugar during the initial hours, followed by a subsequent decline in consumption. This behaviour may be attributed to product inhibition or a deficiency of essential supplementary nutrients. However, the final results are nearly the same across all tanks (Figure 1), and it can also be stated that the optimal fermentation time may be around 150 hours, at which point the substrate concentration reaches its minimum value.

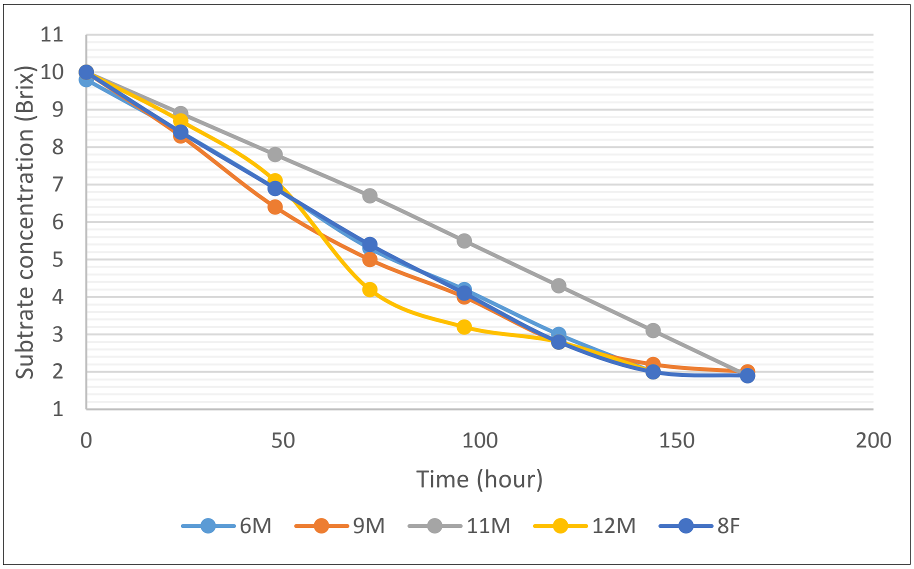


Figure 1. The dependence of the substrate on time.

The dependence of the temperature on time

The fermentation process can also be analysed by studying the dependence of temperature on time because temperature significantly influences the process's

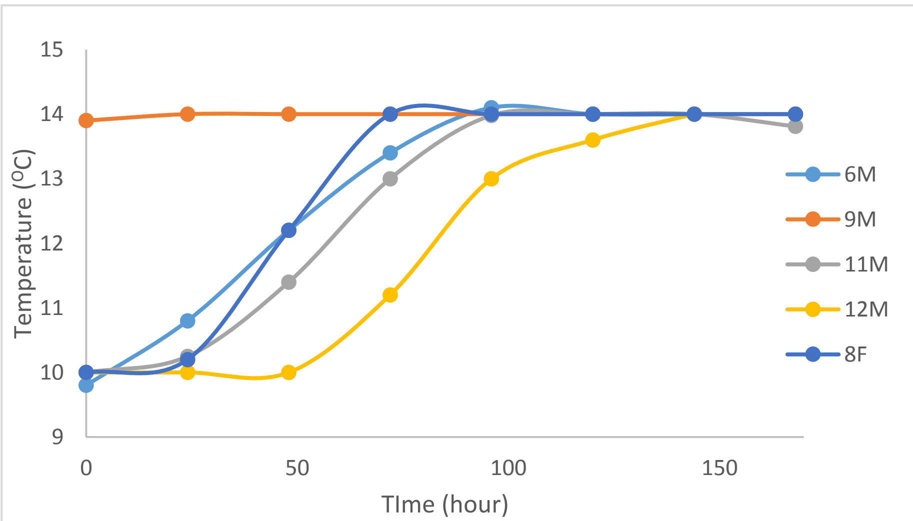
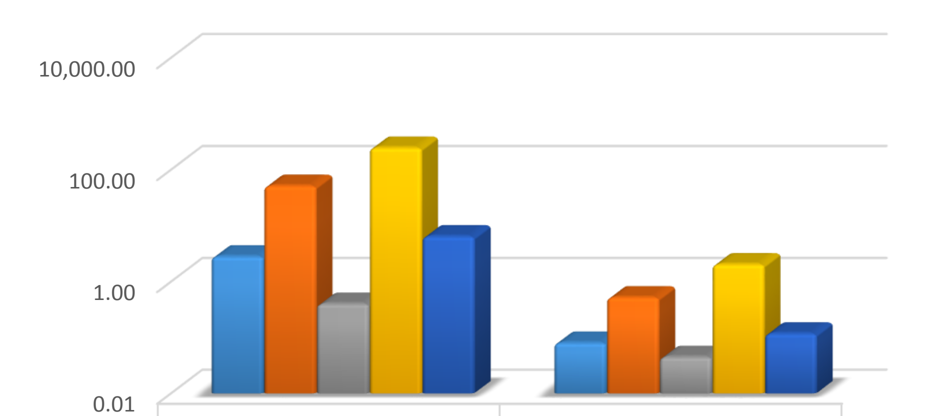


Figure 2. The dependence of the temperature on time.

kinetic constants. In the four processes, the fermentation started at 10 ° C, except for the second tank, where it started at 14 ° C, and all the fermentations ended at 14 ° C.
From Figure 2, the progress of the dependence of the substrate in the above paragraph is better understood because it did not exceed the critical temperature of the yeast, and it was worked at the optimal temperature for effective development of the fermentation process. The temperature varies depending on the extract amount, the yeast's vitality, and other factors.

Determination and comparison of kinetic constants

Kinetic constants such as the half-saturation constant K_s and the maximum growth rate μ_{max} were determined through Lineweaver-Burk graphical linearisation. Microorganisms require energy to break down substrates with long carbon chains, so the specific rate for simpler substrates is more significant than that for more complex substrates, providing optimal



	Ks (Brix)	µmax (1/hour)
6M	2.8185	0.0805
9M	51.4175	0.5229
11M	0.4013	0.0447
12M	246.5443	2.0387
8F	6.4539	0.1178

Figure 3. The values of kinetic parameters

conditions for development. It is noted that both of these kinetic parameters are outside the variations of the theoretical values because the process was developed at different temperatures, focusing on the feature of beer production on an industrial scale.

The obtained values show that the semi-saturation constant decreases as the microorganism's maximum growth rate declines. Based on these values, it is observed that the half-saturation constant exhibits more favourable results in tanks 11M, 6M, and 8F, where a lower substrate concentration is needed to reach half of the maximum growth rate. In contrast, tanks 9M and 12M demonstrate significantly higher values, indicating that a greater substrate concentration is required to achieve growth rates within acceptable limits. These indicators support the trends in Figure 1, where the fermentation process progressed more effectively in tank 11 M. In the fourth tank, the temperature is maintained constant for half the time during fermentation.

Let us compare and interpret the values obtained from the maximum specific growth rate calculations. It can be concluded that the highest rate is observed in tank 12M, followed in decreasing order by tanks 9M, 8F, 6M, and 11 M. This suggests that tank 12M has a greater potential for cellular growth compared to tank 11M, provided that the substrate concentration remains at normal levels.

Furthermore, during this same tank, a more significant decrease in the substrate amount is observed. All these parameter variations arise from developing the process at different temperatures within the defined interval.

In conclusion, it can be stated that tanks 6M and 8F are the most favorable for the development of the fermentation process, based on the values of the constants obtained from the calculations. This conclusion is drawn by evaluating both constants simultaneously, rather than assessing them individually.

Comparison of mathematical models

After determining the kinetic constants, we can build mathematical models with Excel and Matlab. Three mathematical models were studied for the five fermentations carried out in industrial conditions.

The theoretical and Experimental models (Figures 4,5, 6,7, and 8) are graphically presented below.

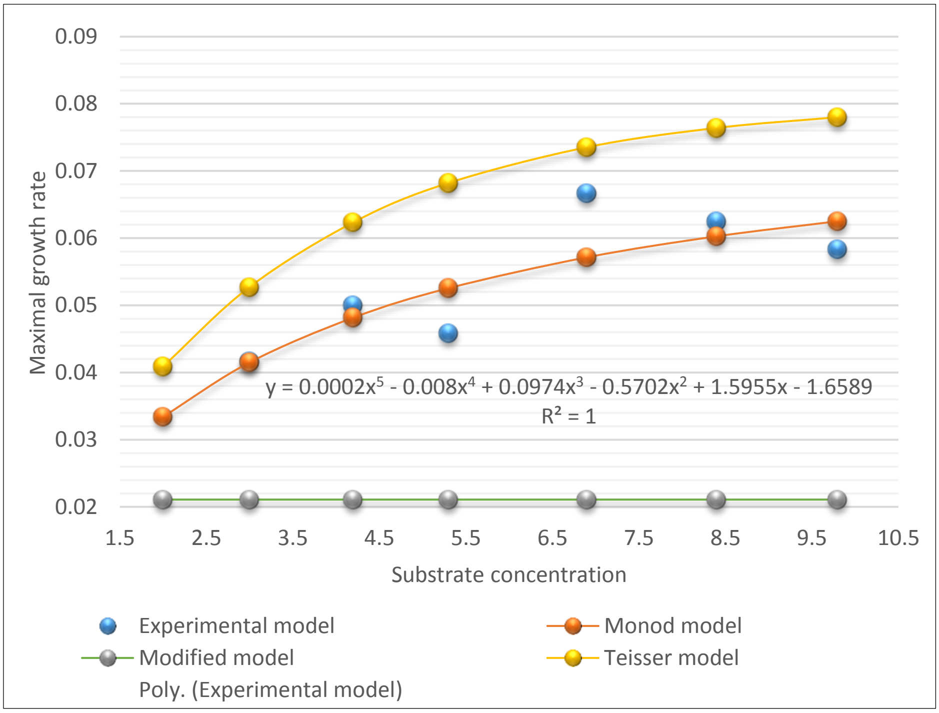


Figure 4. Comparison of mathematical models in the first fermentation process

Based on the obtained graphs, we can observe the progress of the fermentation process, indicating that industrial fermentation differs from theoretical fermentation. The modified Monod model does not align with any of the experimental models. For the five tanks, compatibility is notably high with the mathematical model in the polynomial form of the fifth or sixth order.

However, theoretical models such as Monod and Teisser are not ruled out, as the average compatibility for the five tanks with the Monod model is 49.2%. In contrast, the Teisser model stands at 50.1%. It is important to highlight only the third tank, which deviates more than the others from the theoretical models because the fermentation process has developed under conditions of a gradual temperature increase, unlike the other tanks.

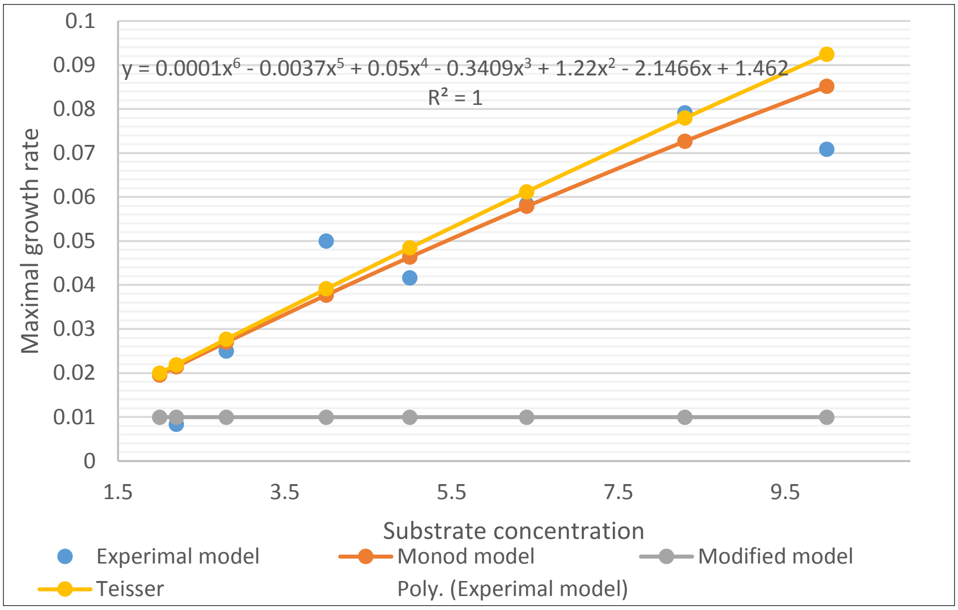


Figure 5. Comparison of mathematical models in the second fermentation process.

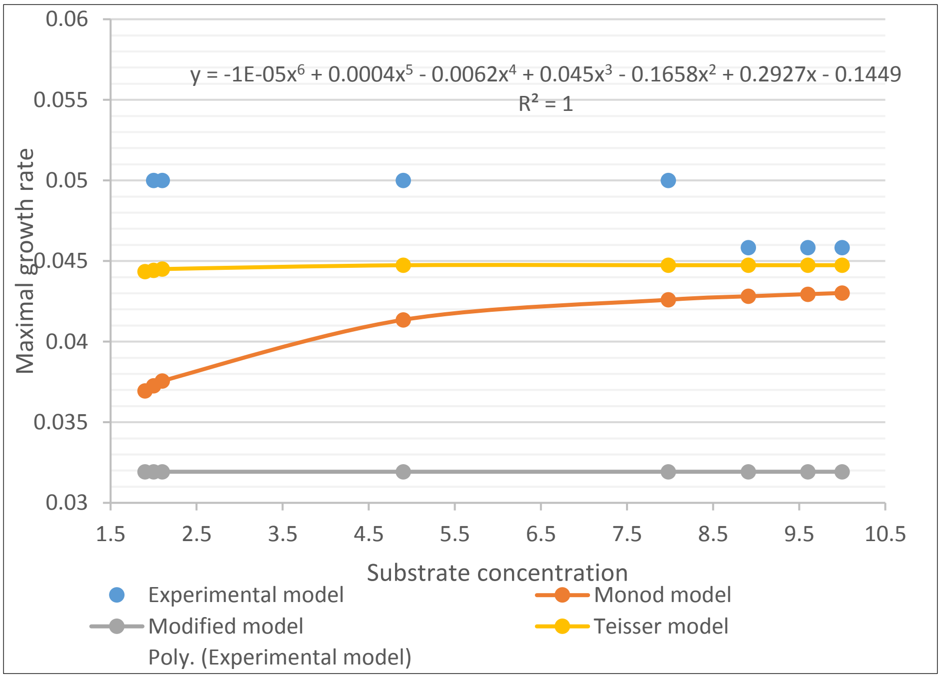


Figure 6. Comparison of mathematical models in the third fermentation process.

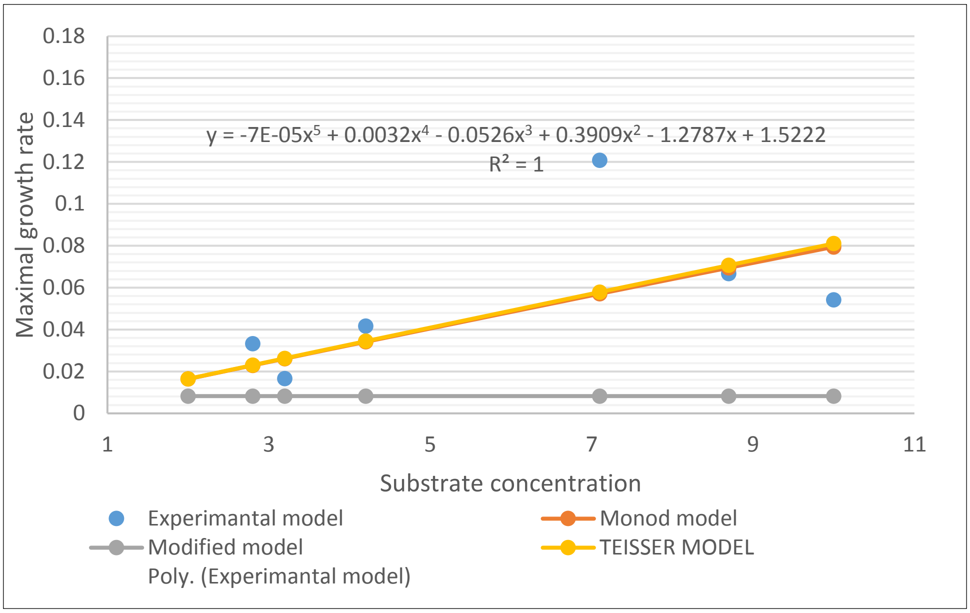


Figure 7. Comparison of mathematical models in the fourth fermentation process.

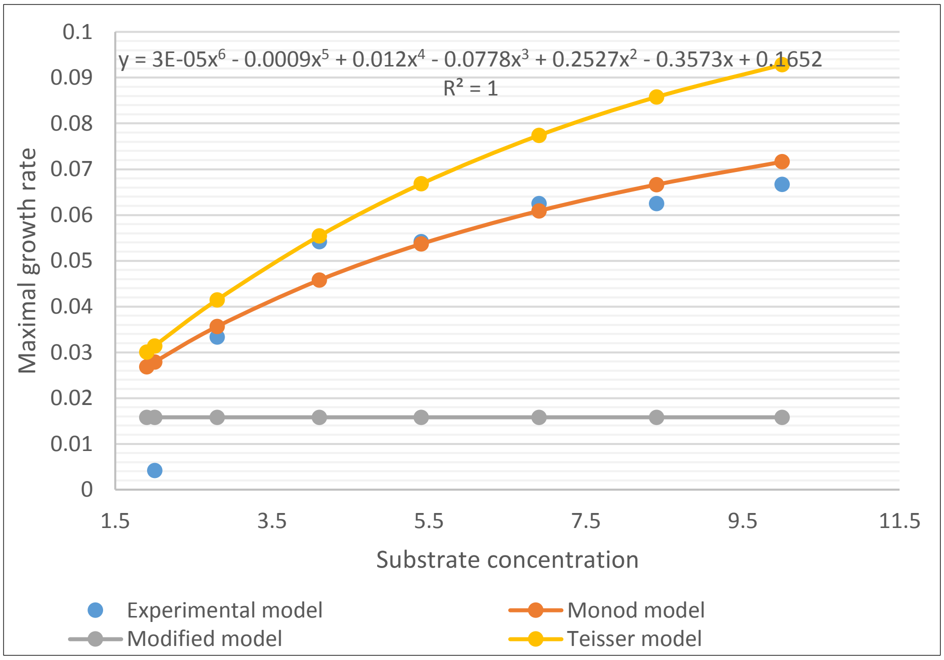


Figure 8. Comparison of mathematical models in the fifth fermentation process.

Considering the characteristics of each theoretical model and comparing them with the experimental model, it can be concluded that the Teissier model aligns more closely with the experimental data. This is due to the Teissier model's ability to adapt well to both low and high concentrations, as well as to conditions where growth is non-linear. Furthermore, the Teissier model is more advanced in capturing non-linear behaviours, whereas the Monod model is classical, where the relationship must always remain hyperbolic.

Conclusions

This research studied the periodical fermentation process with free yeast *Saccharomyces carlsbergensis* under industrial conditions. In general, the fermentation process progresses the same way. It starts at 10^{O}Brix and ends at approximately 2^{O}Brix. However, it was noticed that the process progresses better when carried out at the optimal temperature of 10-14^{O}C, increasing gradually. In this way, the amount of substrate decreases over time. All this comes from the significant impact of temperature on the microbial processes that develop. All our interest consists of determining kinetic parameters and evaluating mathematical models to use them in improving processes and equipment.

In the fermentation processes, it is observed that the maximum growth rate μ_{max} is the same, only in the fourth tank it is approximately 90% greater than the average of the other tanks because the temperature increase was made after 72 hours, and this has effected in obtaining a more considerable value of the parameter. The specific growth rate depends on the type of microorganism and the fermentation conditions; the latter is indicated by the values obtained. From the obtained values, we noticed that with the reduction of the maximum growth rate of the microorganism, the half-saturation constant decreased.

Based on the values of the constants obtained from the calculations, tanks 6M and 8F are the most favorable for developing the fermentation process. This conclusion is drawn by evaluating both constants simultaneously rather than assessing them individually.

The high values of the kinetic parameters are explained by the fact that the fermentation process was developed with free yeast, where the transport of metabolites into the cell and the exit of products outside is much easier due to diffusion through the cell membrane.

Based on the graphs obtained, we can see the progress of the fermentation process, where it is noted that industrial fermentation differs from the progress of theoretical fermentation. The modified Monod model does not match any of the experimental models. For the five tanks, it is noted that the compatibility is very good with the mathematical model in the polynomial form of the fifth or sixth order. However, theoretical models such as Monod and Teisser are not excluded, where the average compatibility for the five tanks with the Monod model is 49.2%, and the Teisser model is 50.1%.

By looking at the features of each theoretical model and comparing them with the experimental results, we can say that the Teissier model fits the data better.

Given the values of kinetic constants, the performance of the substrate during the fermentation process, and the predictions of theoretical and experimental mathematical models, selecting an appropriate model is complex.

References

Arellano-Plaza, M., Herrera-López, E. J., Díaz-Montaño, D. M., Moran, A., & Ramírez-Córdova, J. J. (2007). "Unstructured kinetic model for Tequila batch fermentation". International Journal of Mathematics and Computers in Simulation.

Bailey, J. E., & Ollis, D. F. (1986). Biochemical engineering fundamentals (2nd ed.). McGraw-Hill Book Company.

Di Serio, M., Tesser, R., & Santacesaria, E. (2001). A kinetic and mass transfer model to simulate the growth of baker's yeast in industrial bioreactors. Chemical Engineering Journal, 82,347–354. http://www.ingentaconnect.com/content/tandf/bsp/2002/00000013/00000007/art00004

Doran, P. M. (1997). Bioprocess engineering principles. Academic Press. ISBN: 0122208552

Kostov, G., Popova, S., Gochev, V., Koprinkova-Hristova, P., Angelov, M., & Georgieva, A. (2012). Modeling of batch alcohol fermentation with free and immobilised yeasts Saccharomyces cerevisiae 46 EVD. Biotechnology & Biotechnological Equipment, 26(3), 3021–3030.

Manaj, H., Pinguli, L., Malollari, I., & Dhroso, A. (2018). Impact of temperature on beer fermentation kinetics. UBT International Conference, 172.

Nanba, A., Nishizawa, Y., Tsuchiya, Y., & Nagai, S. (1987). Kinetic analysis for batch ethanol fermentation of Saccharomyces cerevisiae. Biochemical Engineering Journal. https://doi.org/10.1016/0385-6380(87)90088-4

Pinguli, L., Malollari, I. (2022). Inxhinieria e proceseve biokimike (pp. 167–218). ISBN: 978-9928-173-86-7.

Stewart, G. G., & Russell, I. (1986). One hundred years of yeast research and development in the brewing industry. Journal of the Institute of Brewing, 92, 537–558.

Shahida Anusha Siddiqui, Zeki Erol, Jerina Rugji, Fulya Taşçı, Hatice Ahu Kahraman, Valeria Toppi, Laura Musa, Giacomo Di Giacinto, Nur Alim Bahmid, Mohammad Mehdizadeh & Roberto Castro-Muñoz (2023). “An overview of fermentation in the food industry ‑ looking back from a new perspective” Siddiqui et al. Bioresources and Bioprocessing,

https://doi.org/10.1186/s40643-023-00702-y

Terkida, V., Xhagolli, L., & Malollari, I. (2017). Kinetic modeling of immobilised yeast batch fermentation. International Journal of Engineering Research & Sciences, 3(6), 49–55. https://doi.org/10.25125