

Araştırma Makalesi / Research Article

Investigation of Yeast Cells Life Cycle Parameters by Using Multi-Layer Perceptron Artificial Neural NetworkEyyüp GÜLBANDILAR¹, Serel ÖZMEN AKYOL², Aysel GÜLBANDILAR³, Gıyasettin ÖZCAN⁴, Necati KARAKUŞ⁵¹ Eskisehir Osmangazi University, Faculty of Engineering and Architecture, Department of Computer Engine., Eskisehir.² Eskisehir Osmangazi University, Sivrihisar Vocational School, Department of Computer Programming, Eskisehir³ Eskisehir Osmangazi University, Faculty of Agriculture Department of Food Engineering, Eskisehir.⁴ Uludag University, Faculty of Engineering, Department of Computer Engineering, Bursa.⁵ Kutahya Dumlupınar University, Institute of Science and Technology, Kutahya.

*Sorumlu yazar e-posta: egulbandilar@ogu.edu.tr

sozmen@ogu.edu.tr.

aysel.gulbandilar@ogu.edu.tr.

gozcan@uludag.edu.tr.

necatikarakus27@hotmail.com

ORCID ID: <https://orcid.org/0000-0001-5559-5281>ORCID ID: <https://orcid.org/0000-0002-5344-4065>ORCID ID : <https://orcid.org/0000-0001-9075-9923>ORCID ID: <https://orcid.org/0000-0002-1166-5919>ORCID ID: <https://orcid.org/0000-0002-8633-2385>

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Abstract

Examining the growth parameters of yeast cells in the food industry causes to increase both time and labor costs. Simulation models can be put forward to reduce these costs. In this study aimed that design a simulation model for growth cycle parameters of *Saccharomyces cerevisiae* by using the Multi-Layer Perceptron Neural Network (MLPNN). While cultivation time is defined as input parameter in this model, the cell count per hour and growth rate is determined as output parameters. In the designed model, two hidden layer back propagation neural networks are preferred. The first hidden layer uses 10 nodes, while the second hidden layer uses 2 nodes. For the training of this model, 144 experimental data are used, whereas 72 of these experimental data were used for testing the trained model. The developed model showed a high correlation on the growth curve and growth rate in the process both training and test ($R^2_{\text{training}}=0.9993$ and $R^2_{\text{test}}=0.9993$ for growth curve; $R^2_{\text{training}}=0.9381$ and $R^2_{\text{test}}=0.9404$ for growth rate). The results show that developed model can be used successfully in cell culture studies instead of experimental studies in food industry.

Keywords

Saccharomyces cerevisiae;
Growth curve;
Growth rate;
Artificial neural network

Çok Katmanlı Perceptron Yapay Sinir Ağı Kullanılarak Maya Hücrelerinin Yaşam Döngüsü Parametrelerinin Araştırılması**Öz**

Gıda endüstrisinde maya hücrelerinin büyüme parametrelerinin incelenmesi hem zaman hem de işçilik maliyetlerinde artışa neden olmaktadır. Bu maliyetleri azaltmak için simülasyon modelleri öne sürülebilir. Bu çalışmada Çok Katmanlı Perceptron Sinir Ağı (MLPNN) kullanarak *Saccharomyces cerevisiae*'nin büyüme döngüsü parametreleri için bir simülasyon modeli tasarlanması amaçlanmıştır. Bu modelde, ekim zamanı girdi parametresi olarak tanımlanmışken, saatteki büyüme oranı ve hücre sayısı çıktı parametreleri olarak belirlenmiştir. Tasarlanan modelde iki gizli katmanlı geri yayımlı sinir ağı tercih edilmiştir. İlk gizli katmanda 10 düğümün kullanılırken ikinci gizli katmanı 2 düğüm kullanılmıştır. Modelin eğitimi için 144 deneysel veri kullanılırken, bu deneysel verilerin 72'si eğitilmiş modeli test etmek için kullanılmıştır. Geliştirilen model, büyüme eğrisi ve büyüme hızı için hem eğitim hem de test aşamasında yüksek bir korelasyon göstermiştir (büyüme eğrisi için, $R^2_{\text{training}} = 0,9993$ ve $R^2_{\text{test}} = 0,9993$; büyüme oranı için $R^2_{\text{training}} = 0.9381$ ve $R^2_{\text{test}} = 0.9404$). Sonuçlar, geliştirilen modelimizin gıda endüstrisinde deneysel çalışmaların yerine hücre kültürü çalışmalarında başarılı bir şekilde kullanılabilceğini göstermektedir.

Anahtar Kelimeler

Saccharomyces cerevisiae;
Büyüme eğrisi;
Büyüme oranı;
Yapay sinir ağları

1.Introduction

To investigate the growth cycles of microorganisms, cell counts are observed at the time after the cultivation of the cells in the food industry. Continuously cell counting leads to an increase both in terms of time and labor costs. To reduce these costs, mathematical equation models were firstly expressed. One of the simplest model that tend in geometric progression of population growth is presented by Thomas Malthus in the end of the eighteenth centuries. He thinks that the increase in geometric progression of the population growth would sooner or later becomes inadequate in the means of subsistence (Gulbandilar 2005, Gulbandilar et al. 2016, S.Cerevisiae 2017).

Within the life cycle, the yeast cell numbers can be plotted against time. This chart is called the growth curve. This growth curve of yeast cells can be expressed in mathematical model equations. The first description of the mathematical model of this S-shaped growth curve was made by Verhulst. This mathematical form is called the Verhulst logistic equation,

$$N(t) = \frac{N_s}{1 + k \cdot e^{-\mu \cdot t}} \quad (1)$$

where N_s is the number of cells in saturation duration, μ is the growth rate, t is the time, N_0 is the number of cells added and a constant value which is $k = \frac{N_s - N_0}{N_0}$ (Gulbandilar 2005, Gulbandilar 1996). A different mathematical model point of view is the Gompertz equation organized by Zwietering et al.

$$N(t) = A + C \cdot \exp [- \exp [-B(t - M)]] \quad (2)$$

where A is constant, C is number of cell at the end of logarithmic phase, B is the relative growth rate at M time and the M is the time when microorganisms reach the maximum growth rate (Simon and Kariml 2001, Garcia-Gimeno et al., 2002).

In recent years, expert systems have become common in studies relating cell culture analysis. Simon and Kariml (2001) have shown that the growth cycle of the *Bacillus subtilis* can be estimated probabilistic neural networks (PNN).

Similarly, the prediction of the growth cycle of *Escherichia coli*, *Aeromonas hydrophila*, *Shigella flexneri* and *Brochothrix thermosphacta* microorganisms has been studied by using PNN (Jeyamkondan et al. 2001, Hajmeera and Basheer 2002). Garcia-Gimeno and his colleagues (2002) used both artificial neural networks and genetic algorithm methods to determine the growth cycle of food microbial. From a different perspective, Hajmeer et al. (2000) performed a study on the modeling of the growth curve of *Escherichia coli* bacteria with artificial neural networks.

Vasquez et al. (2018) presented a relationship between spectral profiles and hardness values by using two types of regression models which is the partial least squares regression (PLSR) and artificial neural networks (ANN). In their model, The ANN models showed slightly better performance when compared to the PLSR. As a consequence of their yields, (HSI+ANN) was able to predict the texture properties of Swiss-type cheeses throughout the ripening period, where correlation coefficients are respectively 0.95 and 0.96. Another recent study, Veys et al. (2016), studied the growth modelling of the *Salmonella* and *Escherichia coli* on lettuce at different temperatures by using Baranyi's DMFit model and Ratkowsky equation. They showed that their models were suitable to assess the growth rate of both *Salmonella* and *E. coli* on lettuce, that is stored at 5 to 37°C and 10 to 37°C. According to their evaluation results their R^2 values are between 0.92 and 0.99, respectively.

Another study, which was presented by Garcia-Camacho et al. (2016) used feed-forward back-propagation neural network (FBN). In the study, they presented a tool for predicting the growth dynamics of the microalga *Karlodinium veneticum*, which is a culture medium with specified concentrations of the key nutrients. They have found that FBN was an excellent tool for predicting the growth curves in the range of culture conditions. Another study, owned by Pappu et al., (2016) analyzed two models multiple linear regression, MLR, and artificial neural network, ANN, to describe the growth of *Spirulina platensis* outdoor cultures. Their results denoted that that ANN models had a slightly smaller error rate when

compared to MLR models (R^2 values for ANN are between 0.91 and 0.98).

The realization of the growth pattern of the *Saccharomyces cerevisiae* yeast cell, which is widely used in the food sector, is very important both for academic and industrial applications.

During our literature survey, we could not find any study on the use of artificial intelligence methods that predict the growth curves and growth rates of microorganisms jointly.

We needed to consider the joint case since we observed that when the reproduction conditions of microorganisms change, the recalculation of the equation coefficients of the growth curve will take a long time compared with artificial intelligence methods. Thus, making the studies in this growth pattern reduces both the experimental costs and the labor costs. It will also improve the quality of food production. In this study, it is aimed to give a different perspective to the mathematical modeling studies of the growth curve and growth rate of *Saccharomyces cerevisiae* yeast cell. As mentioned above, the Multi-Layer Perceptron Neural Network (MLPNN) which is an expert modeling method, widely used in this area, is preferred.

2. Material and Method

2.1 Cell culture and counting

In this paper, in our laboratory, *Saccharomyces cerevisiae* was isolated from beaker's yeast (Pak Food Producer Com.). In the Sabouroud's dextrose (SD) agar (Oxoid CM41) at 30°C and pH=5.6±0.2 for a day was incubated dry yeast cells. In Sabouroud's liquid medium (Oxoid CM147) was inoculated which isolated yeast cells. The test tubes containing 6 ml of liquid medium was inoculated using considering approximately equal numbers of cells ($5.48 \pm 0.09 \times 10^5 \text{ml}^{-1}$) from the stock culture obtained and they were placed on to shakers (Nüve SL350). The test tubes incubated in the incubator (Nüve EN400) at 30° C for 29 h at 100 rpm.

In order to the determination of the experimental periods, the adaptation phases of the cells which were the first 6 hours of the incubation period were taken into consideration. For determining the changes which increase in population, incubation

periods were increased up to 29 hours. A total of 24 tubes were inoculated. The experiment was repeated eight times under the same conditions.

Thoma lam was used in the determination of cell counts. The Thoma lam is specific count areas for liquid samples known as Haemocytometric method. Where the cell population negatively affects counting, the samples were diluted (1/10-1/100) using 10% (v/v) acetic acid (Merck) solution. Growth cultures obtained, using Gram staining were tested for morphology and culture purification (Gurgun and Halkman 1990).

The growth rate (μ) of the *Saccharomyces cerevisiae* yeast cell was calculated manually by considering Eq.1 ($t=1$ hours). The growth rate was determined over a period of one hour and was found for all incubation time periods.

2.2 Multi-Layer Perceptron Neural Network

The designed model of Multi-Layer Perceptron Neural Network (MLPNN) is implemented in MatLab Toolbox. In order to train MLPNN model, we selected "time" as input variable, whereas growth rate and cell number corresponding to this time period are determined as output variables (Fig.1). While the training, we used 120 data (5 experimental repetitions, 24 measurements per experiment; $24 \times 5 = 120$) from total 192 cell number data. Therefore, remaining 72 data (3 experimental repetitions, 24 measurements per experiment; $24 \times 3 = 72$) are used to analyze the accuracy of the model. In the designed MLPNN model was chosen feed-forward back propagation which as one of the most popular training algorithms for advanced NNs. There are two hidden layers as shown in Fig.1 in the designed structure. The Levenberg-Marquardt function is used in the system training and the learn_gdm function is used as adaptation learning function. As a transfer function is tansig, MSE-mean squared error is applied as a performance function to evaluate the results (Karakus, 2019).

After the collecting the values of the momentum and determination of the learning rate, 30 iterations are made for the training of the model. The parameters obtained from training of MLPNN model are summarized in Table 1. The 72-input data,

dedicated to the test of the trained model, are given as input only (Karakus, 2019).

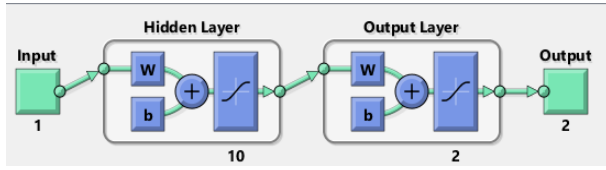


Figure 1. Block diagram of the MLPNN model used to construct the growth curve and growth rate

3. Results

The MLPNN model, which has two hidden layers developed in the study, shows that can provide reliable results. The number of hidden layers and the number of neurons in this layer for obtaining good prediction results in MLPNN models are very important. There is no general rule applied for this number is determined. A specific value can be obtained for each system performance. But in general, starting with a few neurons, the number of neurons in the hidden layer is gradually increased to try to achieve the best result. This process is repeated for each layer. Thus, the structure of the model is created with the MLPNN model remaining within the acceptable error range. In this study, a similar strategy is applied. The number of hidden layers in the obtained structure is two, and the number of neurons in the first hidden layer is ten and the number of neurons in the second hidden layer is two.

Table 1. Parameter values used in model

Parameters	MLPNN model
Number of neurons in the input layer	1
Number of hidden layers	2
The number of neurons in the first hidden layer	10
Number of neurons in the second hidden layer	2
The number of neurons in the output layer	2
Error in training result	1x10 ⁻⁷
Epoch	30

The correlation between the developed model and the experimental results are compared by using the coefficient of determination (R^2), the mean absolute percent error (MAPE) and root mean square error (RMS) criteria. MAPE and RMS values can be calculated with the help of Equations (3), (4) and (5).

$$RMS = \sqrt{\frac{1}{N} \sum_{i=1}^N |t_i - O_i|^2} \quad (3)$$

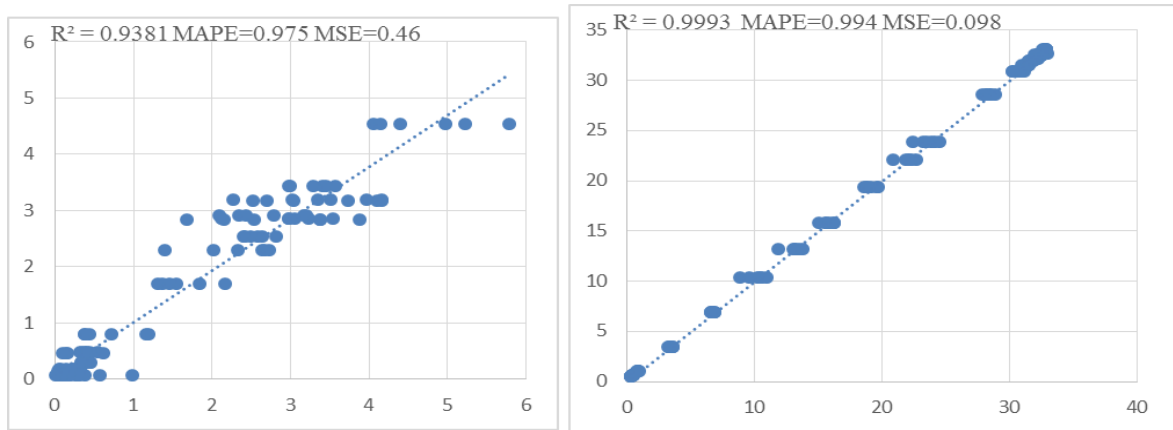
$$R^2 = 1 - \left(\frac{\sum_{i=1}^N (t_i - O_i)^2}{\sum_{i=1}^N (O_i)^2} \right) \quad (4)$$

$$MAPE = \frac{1}{N} \sum_{i=1}^N \left| \frac{(t_i - O_i)}{O_i} \right| * 100 \quad (5)$$

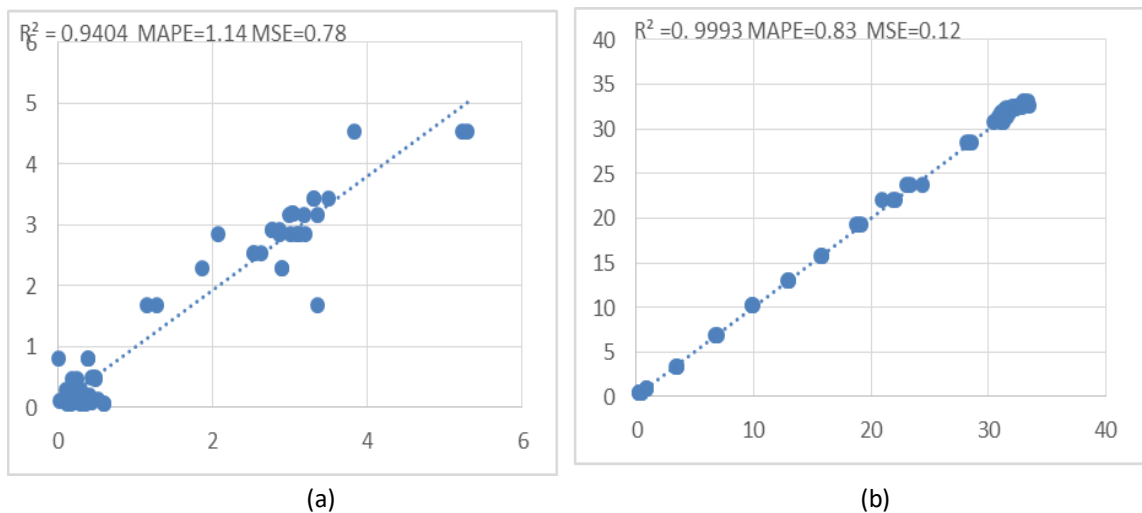
where N is the total number of samples, t is the target value and O is the output value of MLPNN (Ozcan et al. 2009, Dogan and Gonullu, 2018).

While 120 experimental data of total 192 data were used for the training of the MLPNN model, 72 data were selected for testing. The scatter plot between the developed model results and the experimental results is given in Fig.2 and Fig.3. In addition, statistical results obtained from Equations 4, 5 and 6 can be seen on these Figures. When the results of statistical comparison of training and test data are examined, it is seen that there is a high correlation between scatter plot data ($R^2_{\text{training}}=0,9993$ and $R^2_{\text{test}}=0,9993$ for growth curve; $R^2_{\text{training}}=0,9381$ and $R^2_{\text{test}}=0,9404$ for growth rate). In addition, the error rate between the experimental and the MLPNN groups seems to be very low.

The mean cell numbers versus time for the results obtained by developed MLPNN model with the experimental results of 72 data used for the test are given in Fig.4. The similarity between the mean cell number of the data obtained from the MLPNN model and the mean changes of the experimental results is also shown in Fig.4.



(a) (b)
Figure 2. Growth rate (a) and growth curve (b) for training of developed model



(a) (b)
Figure 3. Growth rate (a) and growth curve (b) for testing of developed model

4. Conclusion

This study presented a new Multilayer Perceptron Neural Network model for growth cycle parameters of *Saccharomyces cerevisiae*. Contribution of the study is presented in twofold aspects. First and main contribution of the study is concerning with microorganism. To the best of our knowledge, this is the first study that investigated *Saccharomyces cerevisiae* in literature.

Our second contribution is about analysis methodology. While studies on the literature either investigates growth rate or growth curve of the microorganisms, our study analyzes both rates jointly.

The performance results of the study is also effective. Our prediction results outperforms, in terms of R^2 test. We have presented the effective performance by means of MAPE and RMS as well. In terms of MLPNN model results, we observed that explained model produces successful results during the determination of growth curve and growth rate of *Saccharomyces cerevisiae* yeast cell.

Developed neural network model can be used to in the industry. So that food quality can be enhanced by means of prediction of *Saccharomyces cerevisiae* growth. Consequently, due to reliable predictions, labor costs of food sector can be reduced. Finally food production costs can be depleted.

Experimental working conditions are demonstrated the limitations of our model as well. These limitations are ambient temperature, pH value and a certain number of starting cells. In future work, the above limitations will be repeated for different values. Thus, our data pool will be enriched.

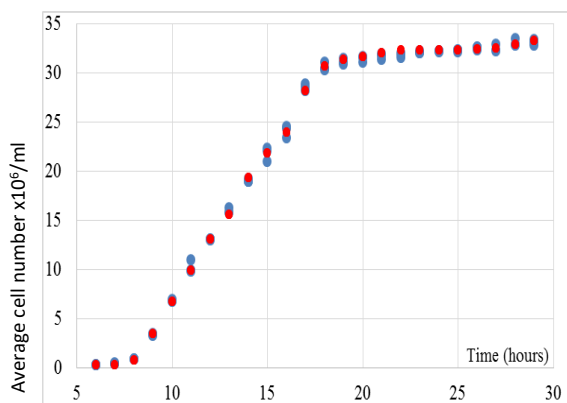


Figure 4. Experimental and MLPNN model results change over time

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