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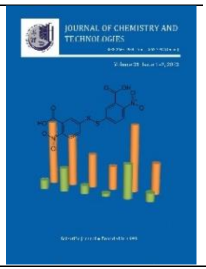

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OPTIMIZATION OF THE PARAMETERS OF BIOCATALYTIC HYDROLYSIS OF VEGETABLE OIL USING THE METHODS OF NEURAL NETWORKS AND GENETIC ALGORITHMS

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Abstract

At present, ensuring the competitiveness of domestic oil and fat products on the domestic and foreign markets should be implemented at the expense of knowledge-intensive production based on the introduction of innovations that provide a qualitatively new level of technological development. In this regard, in the presented work, research was carried out on the optimization of the parameters of biocatalytic hydrolysis of sunflower oil. NovoCor AD L (Novozymes, Denmark) enzyme preparation was used as a biocatalyst. The mathematical apparatus of artificial neural networks was used for the process modelling. The three-layer direct signal transmission network developed as a result of construction, learning and verification was used to calculate the fitness function in the optimization of biocatalytic hydrolysis using genetic algorithms. The software implementation of the mathematical apparatus was performed in the MATLAB environment. The conducted research made it possible to establish the optimal values of the main parameters of the biocatalytic hydrolysis of vegetable oil: the molar ratio of water to oil is 14 : 1, the amount of enzyme is 3.75 % in relation to the mass of oil, the temperature is 60 °C, and the reaction time is 480 minutes. The set optimal parameters were tested in the conditions of experimental and industrial production. According to the test results, the degree of oil hydrolysis was 96 ± 1.8 %, which correlates well with the modelling data.

Keywords: hydrolysis; lipase; biocatalysis; artificial neural network; genetic algorithm.

ОПТИМІЗАЦІЯ ПАРАМЕТРІВ БІОКАТАЛІТИЧНОГО ГІДРОЛІЗУ РОСЛИННОЇ ОЛІЇ З ВИКОРИСТАННЯМ МЕТОДІВ НЕЙРОННИХ МЕРЕЖ І ГЕНЕТИЧНИХ АЛГОРИТМІВ

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Анотація

В теперішній час забезпечення конкурентоспроможності вітчизняної олійно-жирової продукції на внутрішньому та зовнішніх ринках повинне бути реалізоване за рахунок наукоємного виробництва на основі впровадження інновацій, які забезпечують якісно новий ступінь розвитку технологій. У зв'язку із цим у представленій роботі виконано дослідження щодо оптимізації параметрів біокаталітичного гідролізу соняшникової олії. В якості біокаталізатора був використаний ферментний препарат NovoCor AD L («Novozymes», Данія). Для моделювання процесу було застосовано математичний апарат штучних нейронних мереж. Розроблена в результаті конструювання, навчання і верифікації тришарова мережа прямої передачі сигналу використовувалась для обчислення функції пристосованості при оптимізації біокаталітичного гідролізу методом генетичних алгоритмів. Програмну реалізацію математичного апарату було виконано у середовищі MATLAB. Проведені дослідження дозволили встановити оптимальні значення основних параметрів біокаталітичного гідролізу рослинної олії: мольне співвідношення води до олії – 14 : 1, кількість ферменту – 3.75 % по відношенню до маси олії, температура – 60 °С, час реакції – 480 хвилин. Встановлені оптимальні параметри були апробовані в умовах дослідно-промислового виробництва. Відповідно до результатів випробувань ступінь гідролізу олії складав 96 ± 1.8 %, що добре корелюється із даними моделювання.

Ключові слова: гідроліз; ліпаза; біокаталіз; штучна нейронна мережа; генетичний алгоритм.

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Introduction

One of the large-scale productions of the oil and fat industry is the production of fatty acids by hydrolysis of fatty raw materials. Millions of tons of fatty acids are produced annually in the world, which are used as a food additive, foam stabilizer, glazing agent and foam extinguisher, they are also a raw material for the synthesis of biodiesel and surfactants [1]. Among all fatty acids, a special place belongs to unsaturated acids. These substances are widely used in the food industry as a substrate for the synthesis of health-promoting compounds [2].

Fat hydrolysis is a catalytic process. Catalysts are hydrogen ions and hydroxide ions, they are either introduced into the fat-water system by adding acids or other substances, the dissociation of which leads to the creation of H^+ and OH^- ions, or they increase their concentration, creating conditions for increasing the degree of water dissociation [3].

The existing methods of fat hydrolysis are associated with the use of toxic catalysts (acids, alkalis), high temperatures (200–225 °C) and pressure (2.0–2.5 MPa), which does not allow obtaining unsaturated fatty acids of high degree of purity due to the processes of polymerization and oxidation [4; 5].

One of the ways to avoid these defects is to replace chemical processes with biocatalytic ones. Biocatalytic processes take place in mild conditions and do not require complex hardware design [6–9]. All this ensures a significant reduction in material and energy costs by increasing the output of high-quality target products along with minimizing the formation of byproducts [10–13].

Lipases are the main biocatalysts used for the conversion of fatty raw materials [14]. Lipases (triacylglycerol hydrolases) are versatile enzymes used for conversion of lipids [15]. In recent years, researches have been carried out in Ukraine on the use of these biocatalysts in the processes of glycerolysis and interesterification of fats [16–18].

Lipases of vegetable, animal and microbial origin are used for the hydrolysis of fats [19; 20]. In practice, the lipases, the producers of which are various microorganisms, are most often used [21; 22]. For example, Pseudomonas fluorescens, Rhizopus niveus, Thermomyces lanuginosa, Rhizopus delemar, Geotrichum candidum etc. [23].

The hydrolysis reaction takes place on the surface of the fat-water phase distribution and is determined by four main factors: the ratio of the starting substrates, the amount of enzyme, temperature and time [24, 25]. At the same time, a reduced water content leads to a low degree of fat hydrolysis, and an excess leads to a decline in the reaction rate due to a decrease in the concentration of the enzyme. Considering the relatively high cost of the enzyme preparation, it is necessary to minimize its amount in the reaction mixture, while ensuring the flow of the biocatalytic process with the maximum yield of reaction products. This process is endothermic, i.e. it requires the introduction of heat. At the same time, beyond the temperature limit of lipase stability, the positive effect of temperature increase due to the protein nature of the enzyme is to some extent compensated by the negative effect of thermal denaturation. The point of full compensation is the optimal temperature for enzyme action. It should be noted that the optimum time for biocatalytic hydrolysis is determined by ensuring the maximum degree of hydrolysis possible under the given conditions and economic feasibility.

The aim of the study was to establish the optimal parameters of biocatalytic hydrolysis of vegetable oil using the maximum degree of its hydrolysis as a criterion for optimization.

The joint use of artificial neural networks and genetic algorithms in the processing of experimental data became a tool for achieving this goal. This method is one of the most effective mathematical tools for optimizing complex multiparameter functional dependencies [26–28].

Artificial neural networks are mathematical models, as well as their software or hardware implementations, built according to the principle of organization and functioning of biological neural networks [29]. Neural networks are a system of connected and interacting simple processors (artificial neurons). These networks are not programmed in the usual sense of the word, they learn. The ability to learn is one of the main advantages of neural networks over traditional algorithms. Technically, learning consists in finding the coefficients of connections between neurons. In the process of learning, a neural network is able to detect complex dependencies between input data and output data, as well as perform generalizations. This means that in the case of successful learning, the network will be able to return the correct result based on data that were missing from the learning sample.

Genetic algorithms are search procedures based on the mechanisms of natural selection and inheritance [30]. They use the evolutionary principle of survival of the fittest individuals.

These algorithms differ from traditional optimization methods in several basic elements. In particular: they process not the values of the parameters of the task itself, but their coded form; search for a solution based not on a single point, but on some of their population; use only the target function, and not its derivative or other additional information; apply probabilistic rather than deterministic selection rules. All of the listed properties determine the fact that at this time the apparatus of genetic algorithms is one of the most modern and best methods of optimizing complex polyparametric and multi-extreme functional dependencies, which include the process of enzymatic hydrolysis.

Experimental part

The model mixtures consisted of sunflower oil (manufactured by Bunge Limited) and water, the amount of which varied from 3 mol to 15 mol per 1 mol of oil. The reaction was catalyzed by the enzyme preparation NovoCor AD L (Novozymes, Denmark). NovoCor AD L is a liquid preparation of microbial non-specific lipase type A of Candida antarctica. The amount of biocatalyst was from 0.5 % to 5.0 % in relation to the mass of the oil. The process was carried out at temperatures from 30 °C to 70 °C with continuous stirring under a layer of nitrogen. The reaction time was from 120 to 600 minutes. The choice of the enzyme preparation and the intervals of parameter variation was carried out based on the results of preliminary studies. At certain time intervals, samples were taken, in which the degree of oil hydrolysis (DH, %) was determined according to equation (1):

$$DH = 100\% \cdot \frac{AV}{SV}, \quad (1)$$

where AV is the acid value of the sample, mg KOH/g; SV is the saponification value of the original sunflower oil, mg KOH/g. The analysis of acid values and saponification values was carried out according to the methods [31] and [32], respectively.

The obtained experimental data (the average value of two parallel studies) were used as input for modeling and optimizing the parameters of the biocatalytic hydrolysis process through the combined use of neural networks and genetic algorithms. The software implementation of the mathematical apparatus was performed in the MATLAB environment (The Mathworks, Inc.).

Results and their discussion

Preliminary modeling of the process of biocatalytic hydrolysis consisted in determining the structure of the network, which was carried out by conducting a number of computational experiments with various parameters of the topology – the number of layers, the number of neurons in a layer, the activation function etc. As a result, to approximate the experimental data, we built a three-layer direct signal transmission network with 17 and 11 neurons in the first and second (hidden) layers, respectively, and 1 neuron in the third (output) layer. The structure of the developed network is presented in fig. 1.

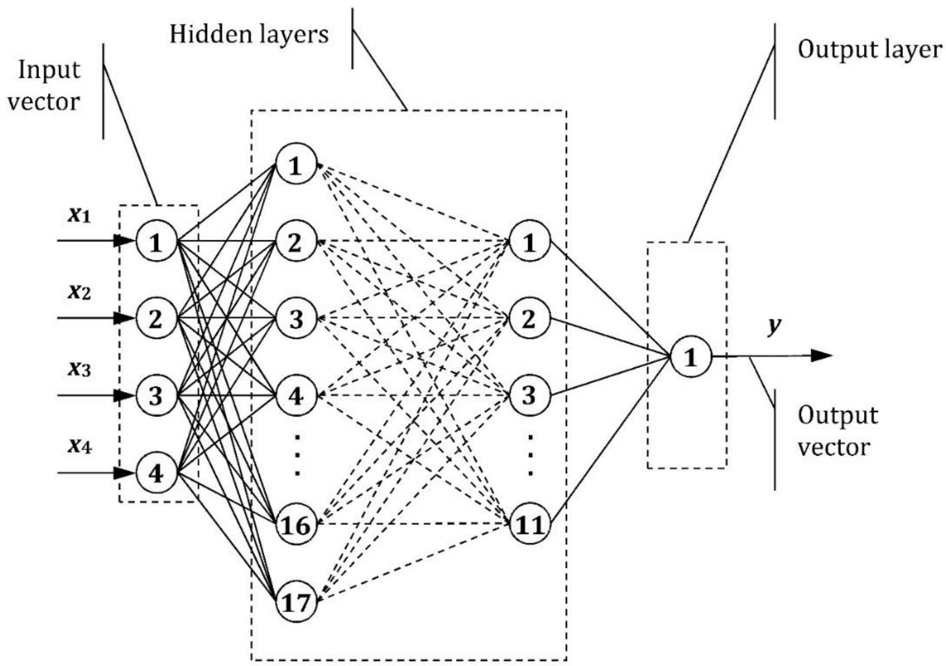

Fig. 1. Scheme of a three-layer direct signal transmission network used to approximate experimental data of the biocatalytic hydrolysis process

The hyperbolic tangent function was chosen as the activation function of the hidden layers and the output layer. A combined quality criterion was used as a function of evaluating the quality of learning. The Levenberg-Marquardt algorithm is used as an adaptation and learning algorithm. The number of learning epochs is 100. Accuracy – 0.0001.

The data from biocatalytic hydrolysis experiments were used for learning and verification of an artificial neural network. The volume of learning and verification samples was equal to 95 and 20 measurements, respectively. Their structure and meaning are presented in Tables 1 and 2, respectively.

Table 1

A fragment of the learning sample

H_2O:oil, mol/mol	The amount of enzyme, % mass	Temperature, °C	Time, min.	Degree of hydrolysis, %		Absolute deviation, %
				Experiment	Model	
3	0.50	30	600	17.0	16.7	1.9
3	5.00	30	600	32.4	32.6	0.6
3	0.50	70	480	19.0	19.2	1.2
3	5.00	50	120	27.5	27.6	0.5
3	5.00	50	480	45.6	44.9	1.5
6	3.75	60	360	45.2	44.5	1.5
6	3.75	40	360	32.9	33.7	2.6
6	3.75	40	600	55.2	53.6	2.9
9	2.50	50	480	49.1	47.3	3.6
9	0.50	50	480	33.0	32.1	2.7
9	1.25	50	540	43.0	43.3	0.8
9	1.25	70	540	41.5	41.0	1.2
9	2.50	40	540	44.5	43.1	3.1
12	0.50	60	240	39.3	40.0	1.9
12	2.50	60	240	61.3	62.2	1.4
12	5.00	60	240	83.0	83.6	0.7
12	5.00	70	120	35.2	34.9	0.8
12	5.00	70	360	53.4	53.6	0.3
12	5.00	70	600	75.9	77.0	1.5
15	1.25	50	360	56.6	57.6	1.9
15	2.50	50	360	73.1	72.8	0.4
15	5.00	50	360	79.2	79.4	0.3
15	2.50	40	540	72.1	72.8	0.9
15	2.50	60	540	89.8	90.2	0.4
15	3.75	60	240	76.2	76.5	0.4

Table 2

Verification sample

H_2O:oil, mol/mol	The amount of enzyme, % mass	Temperature, °C	Time, min.	Degree of hydrolysis, %		Absolute deviation, %
				Experiment	Model	
3	2.50	30	600	22.7	21.8	3.9
3	0.50	70	240	17.3	16.6	3.8
3	0.50	70	600	24.0	23.8	0.9
3	5.00	50	540	49.4	50.8	2.8
6	2.50	60	360	33.7	34.2	1.4
6	5.00	60	360	59.0	58.4	0.9
6	3.75	40	120	21.9	21.6	1.2
6	3.75	40	540	46.0	44.7	2.8
9	1.25	50	480	35.5	35.6	0.4
9	3.75	50	480	64.9	63.7	1.8
9	1.25	40	540	38.0	37.5	1.4
9	1.25	60	540	45.4	45.2	0.4
9	2.50	60	540	55.4	56.3	1.7
9	2.50	70	540	50.3	50.7	0.9
12	1.25	60	240	51.7	51.3	0.8
12	3.75	60	240	79.8	79.1	0.8
12	5.00	70	240	46.3	44.6	3.7
15	3.75	50	360	78.3	80.5	2.9
15	2.50	50	540	80.8	79.6	1.5
15	2.50	70	540	69.0	67.9	1.6

The data given in tables 1 and 2 testify to the adequacy of the neural network to the experimental data. The average value of the absolute deviation of model data from experimental data in the learning sample was 1.4 %, and in the verification sample – 1.8 %.

The multilayer artificial neural network developed as a result of construction, learning and verification was further used to compute the fitness function in the optimization of biocatalytic hydrolysis using genetic algorithms. The following values of the parameters of the genetic algorithm apparatus were set: sample size – 200, number of elite descendants – 20, number of generations – 50. Adaptive and heuristic functions were used as mutation and crossover functions, respectively.

The best (maximum) values of the fitness function on the corresponding generation of the functioning of genetic algorithms are presented in fig. 2.

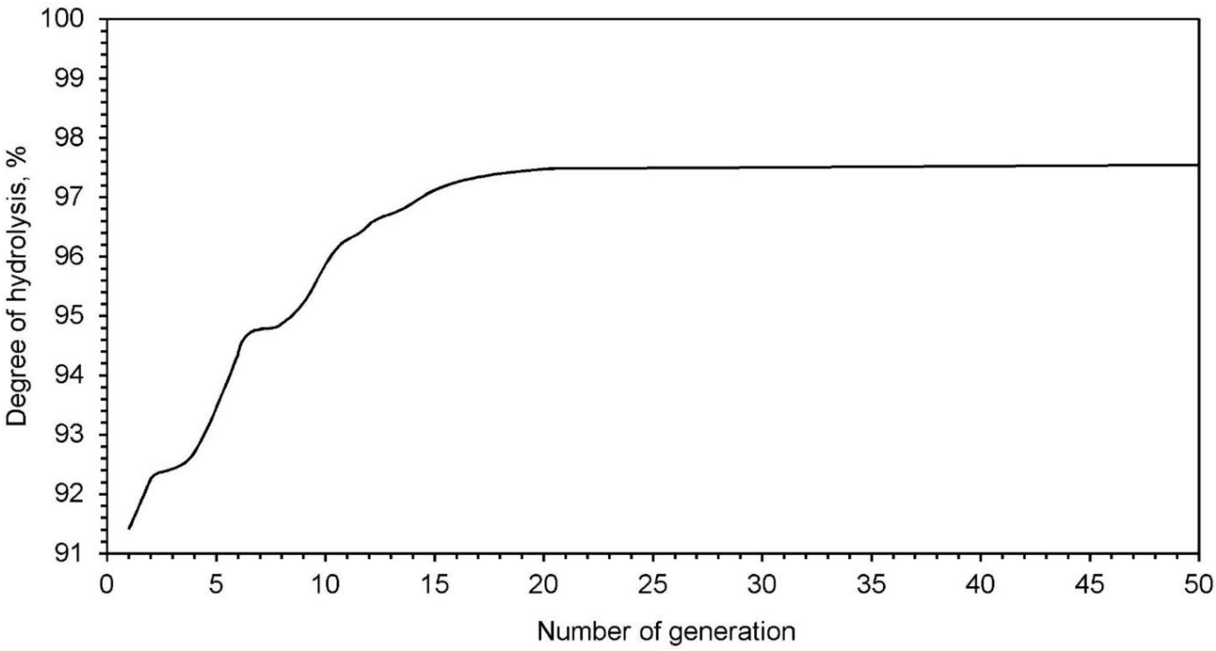

Fig. 2. Optimization process of biocatalytic hydrolysis of vegetable oil

As can be observed (Fig. 2), starting from the 21st generation, the presence of a constant value of the response function is noted, which corresponds to the establishment of its optimum – 97.5 % of the degree of hydrolysis of sunflower oil. This result is achieved with the following calculated values of the initial parameters: the molar ratio of water to oil is 14 : 1, the amount of enzyme is 3.7 5% in relation to the mass of oil, the temperature is 60 °C, and the reaction time is 480 minutes.

The established optimal parameters of biocatalytic hydrolysis were tested in the conditions of experimental and industrial production. According to the test results, the degree of hydrolysis of sunflower oil was 96 ± 1.8 %, which correlates well with the simulation data.

Conclusions

The optimal parameters of biocatalytic hydrolysis of vegetable oil were determined by the method of combined use of genetic algorithms and neural networks. Research and industrial tests confirmed the adequacy of mathematical modeling. The results of the conducted research will serve as a scientific basis for the development of biocatalytic technology for the production of unsaturated fatty acids.

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