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Ann-Based Modeling And Prediction Of Acetic Acid Yield In *Gluconobacter Oxydans* Fermentation Using Dairy Wastewater

Modelado Y Predicción Basados En Redes Neuronales Del Rendimiento De Ácido Acético En La Fermentación De Gluconobacter Oxydans Utilizando Aguas Residuales Lácteas

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ABSTRACT

Acetic acid (AA) is a valuable bioproduct with broad industrial applications in the food, pharmaceutical, and chemical sectors. In this study, *Gluconobacter oxydans* was employed to produce acetic acid using a modified medium containing 12% dairy wastewater as a cost-effective substrate. The effects of glucose concentration, incubation time, and temperature on acetic acid production were evaluated, and the process was modeled using an Artificial Neural Network (ANN) based on a multilayer perceptron (MLP) architecture (3–2–1 structure). The experimental acetic acid yield ranged from 1.01 to 4.68 g/100 mL, values consistent with those reported in the literature for biological fermentation systems. The ANN model achieved low prediction errors (SSE = 0.756 and 0.187 for training and testing, respectively) and demonstrated strong generalization capacity without overfitting. Connection weight and relative importance analyses revealed that incubation time and temperature were the most influential variables affecting yield, while glucose concentration had a secondary effect. These findings confirm the suitability of ANN as a reliable computational tool for modeling and optimizing nonlinear bioprocesses. The integration of machine learning approaches with microbial fermentation can enhance process understanding and support the development of sustainable acetic acid production strategies using industrial by-products.

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Keywords: Artificial Neural Network (ANN), *Gluconobacter oxydans,* Acetic acid; Fermentation, Bioprocess modelling.

RESUMEN.

El ácido acético (AA) es un valioso bioproducto con amplias aplicaciones industriales en los sectores alimentario, farmacéutico y químico. En este estudio, se empleó Gluconobacter oxydans para la producción de ácido acético utilizando un medio modificado con un 12 % de aguas residuales lácteas como sustrato rentable. Se evaluaron los efectos de la concentración de glucosa, el tiempo de incubación y la temperatura en la producción de ácido acético, y el proceso se modeló mediante una red neuronal artificial (RNA) basada en una arquitectura de perceptrón multicapa (MLP) (estructura 3-2-1). El rendimiento experimental de ácido acético osciló entre 1,01 y 4,68 g/100 mL, valores consistentes con los reportados en la literatura para sistemas de fermentación biológica. El modelo de RNA logró bajos errores de predicción (SSE = 0,756 y 0,187 para entrenamiento y prueba, respectivamente) y demostró una gran capacidad de generalización sin sobreajuste. Los análisis de peso de conexión e importancia relativa revelaron que el tiempo de incubación y la temperatura fueron las variables más influyentes en el rendimiento, mientras que la concentración de glucosa tuvo un efecto secundario. Estos hallazgos confirman la idoneidad de las redes neuronales artificiales (RNA) como herramienta computacional fiable para modelar y optimizar bioprocesos no lineales. La integración de técnicas de aprendizaje automático con la fermentación microbiana puede mejorar la comprensión del proceso y respaldar el desarrollo de estrategias sostenibles de producción de ácido acético a partir de subproductos industriales.

Palabras clave: Red neuronal artificial (RNA), Gluconobacter oxydans, Ácido acético; Fermentación, Modelado de bioprocesos.

INTRODUCTION

Acetic acid (AA) is an industrially significant compound with wide-ranging applications across sectors such as cosmetics, pharmaceuticals, food, and textiles. Traditionally, AA has been synthesized through chemical routes; however, these methods raise concerns related to environmental toxicity and the high cost of reagents involved in chemical synthesis when compared to biologically derived alternatives (Tarón-Dunoyer, et al., 2022; Kalck et al., 2020). Consequently, the development of sustainable and eco-friendly biological processes has emerged as a promising alternative for acetic acid production. Fermentation-based approaches offer several advantages, particularly the capacity to utilize food and organic wastes as substrates, which are readily metabolized by microorganisms to produce AA while generating non-toxic residues.

In this context, Gluconobacter species have attracted increasing attention over the past two decades due to their exceptional ability to partially oxidize sugars and alcohols. Among them, *Gluconobacter oxydans* functions as an efficient biocatalyst and has demonstrated notable potential in enhancing the biosynthesis of oxidized metabolites such as AA (Otero-Pérez, et al., 2024; Es-Sbata et al., 2022; Chen et al., 2016). The exploitation of low-cost and renewable substrates—such as organic residues, food industry by-products, and waste from fruit, meat, and dairy processing—represents a viable strategy for reducing production costs and improving process sustainability (Arias Palma, et al., 2021; Pal and Nayak, 2016). However, optimizing acetic acid yield through biological pathways requires careful adjustment of multiple nutritional and physiological parameters. As highlighted by Fasolo et al., (2020), the use of experimental design techniques is crucial for achieving optimal process conditions.

Artificial Neural Networks (ANNs), inspired by the structure and function of the human brain, represent an advanced computational approach capable of modeling complex, nonlinear relationships (Agatonovic-Kustrin & Beresford, 2000). Owing to their capacity for adaptive learning, ANNs have been increasingly applied in materials science and biotechnology to predict and optimize system behavior where traditional statistical models often fail (Ishtiaq et al., 2024; Ishtiaq et al., 2025). Structurally, an ANN consists of interconnected nodes, analogous to biological neurons, organized into input, hidden, and output layers. These interconnected nodes process signals through weighted connections that are continuously adjusted to minimize prediction errors (Ripley, 2007). The effectiveness of an ANN depends on its architecture and learning rate, which determine its ability to generalize and provide accurate predictions (Pérez-Gomariz et al., 2023; Eltawil et al., 2023).

Recent studies have demonstrated the superiority of ANNs over conventional optimization techniques such as Response Surface Methodology (RSM), particularly in modeling nonlinear and complex phenomena within food processing and engineering contexts (Ameer et al., 2017; Cheok et al., 2012). Unlike statistical models, ANNs fall under the domain of non-statistical machine learning (NSML), enabling the system to learn directly from data patterns and make reliable predictions. Therefore, the present study aims to investigate the influence of glucose concentration, temperature, and incubation time on AA production, and to evaluate the predictive performance of ANNs as a modeling tool for process optimization.

MATERIALS AND METHODS

Cultivation Conditions and Acetic Acid Estimation

The *Gluconobacter oxydans* strain used in this study was obtained from the Microbiology Laboratory of the University of Cartagena, Colombia. The culture was initially activated in Glucose Yeast Carbonate (GYC) broth medium to ensure optimal microbial viability prior to fermentation. For AA production, a modified fermentation medium was formulated containing 12% (v/v) dairy wastewater as a substrate. The medium was sterilized by autoclaving at 121 °C and 15 psi for 15 minutes. The fermentation process was conducted for various days under continuous agitation at 120 rpm, with aeration maintained at a rate of 1 L h^{-1} L^{-1}. Samples were collected daily to monitor AA production. Quantification of AA was performed via acid–base titration following the method described by Sharafi et al., (2010).

ANN-Based Predictive Analysis

ANNs are structured in sequential layers consisting of an input layer, one or more hidden layers, and an output layer. Each neuron in the hidden layer receives a weighted sum of the inputs from the previous layer, and these weights are iteratively adjusted during training to minimize the discrepancy between predicted and actual outputs. Training proceeds until the sum of squared errors reaches a minimum, indicating optimal model convergence. In this study, an ANN model was developed using IBM SPSS Statistics version 24 to predict acetic acid yield under varying process conditions.

The dataset comprised 15 valid observations, all of which were retained for analysis. Data were randomly divided into two subsets: 73.3% (11 cases) were used for network training and 26.7% (4 cases) for testing to evaluate the model's predictive performance. The network architecture included three input variables—glucose concentration, incubation time, and temperature—one hidden layer with two neurons employing a hyperbolic tangent activation function, and a single output neuron corresponding to AA yield.

Prior to training, all input and output variables were standardized to ensure consistent scaling. The performance of the neural networks was evaluated using widely adopted statistical metrics in the field of machine learning as the sum of the squared error.

RESULTS AND DISCUSSION

Estimation of acetic acid

The experimental production of AA ranged from 1.01 to 4.68 g/100 mL, values comparable to those reported by Upadhyay et al., (2023), who observed yields between 0.6 and 2.52 g/100 mL for acetic acid–producing bacteria cultured in GYC medium. In the present study, glucose concentration, temperature, and incubation time were selected as the main independent variables for evaluating their individual and interactive effects on acetic acid production. A total of 15 experimental runs were conducted, and both the experimentally obtained and ANN-predicted AA yields are summarized in Table 1. AA production was assessed using a modified culture medium containing 12% dairy wastewater, sterilized by autoclaving, as a carbon-rich substrate. The results demonstrate that dairy industry effluents can serve as an efficient and low-cost feedstock for AA biosynthesis, aligning with global trends toward waste valorization and circular bioeconomy approaches.

Comparable studies have reported similar production levels using alternative organic substrates. For instance, Fronteras et al., (2021) achieved 4.12 g/100 mL of AA from mango peel fermentation, while Lu et al., (2000) reported the potential of spoiled bananas as a viable carbon source. Likewise, other feedstocks such as corn cob, synthetic media, cloudberry, onion waste, kitchen waste, and spoiled banana have yielded 3.5, 4.32, 5.0, 5.3, 2.5, and 4.36 g/100 mL of AA, respectively (Gong et al., 2019; Iida, 2013; Joung, 2019; Chai et al., 2016). Biological acetic acid production generally occurs through two major pathways. In the first, yeast species convert carbohydrates into ethanol, which is subsequently oxidized to acetic acid. In the second, acetic acid bacteria (AAB) directly oxidize ethanol—produced from carbohydrate metabolism—into AA. During this incomplete oxidation

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process, electrons are transferred to oxygen instead of being fully converted into carbon dioxide, allowing efficient acetic acid accumulation (Cheryan et al., 1997).

Table 1. The design of the experiment of the factors dependent on the AA yield.

Glucose	Temperature	Incubation time	Experimental acetic acid yield	Predictive value ANN
2	25	65	1.61	1.96
5	32.5	65	4.68	4.03
5	25	36	1.01	1.16
5	40	94	2.89	4.17
8	32.5	36	1.11	1.19
2	32.5	94	2.25	2.34
8	40	65	2.35	2.49
5	40	36	1.42	1.18
8	25	65	1.67	1.97
5	25	94	1.96	1.88
2	32.5	36	1.08	1.19
2	40	65	2.28	2.18
5	32.5	65	4.68	4.03
8	32.5	94	2.36	2.40
8	32.5	65	4.68	4.05

ANN predictive modeling

The multilayer perceptron (MLP) neural network developed in this study was designed to model the nonlinear relationship between process variables and AA yield. The model incorporated three input neurons representing the independent variables—glucose concentration, incubation time, and temperature. The network was successfully trained to predict AA production as a function of these experimental parameters. The available dataset was divided into two subsets: 73.3% of the data were used for training and 26.7% for testing the model's predictive performance. The final network architecture followed a 3–2–1 topology, consisting of one hidden layer with two neurons (denoted as H(1:1) and H(1:2)) employing the hyperbolic tangent activation function.

This configuration allowed the network to effectively capture complex nonlinear interactions among the input variables. The output layer contained a single neuron corresponding to the dependent variable—AA yield—and utilized the identity activation function to ensure a linear output response. Bias terms were included in both the hidden and output layers to adjust neuron activation thresholds, thereby enhancing the model's adaptability and overall prediction accuracy. This optimized ANN architecture demonstrated robust learning behavior, confirming its suitability for modeling the biological production of AA under varying fermentation conditions.

Model Performance and Validation

During the training phase, the ANN model achieved a sum of squares error (SSE) of 0.756 and a relative error of 0.151, whereas the testing phase yielded an SSE of 0.187 and a relative error of 0.102. These results demonstrate the model's strong generalization ability and confirm that no overfitting occurred during training. The stopping criterion was satisfied after a single iteration, with no further reduction in error, indicating that the network had reached optimal convergence. Training was completed in less than one second, a negligible computational time attributable to the small dataset size and the compact 3–2–1 network architecture.

The close agreement between predicted and experimental values underscores the accuracy and reliability of the ANN model in estimating acetic acid yield. Overall, these findings confirm that the developed ANN provides a robust and efficient computational tool for modeling and optimizing bioprocesses, particularly for systems involving nonlinear relationships among multiple operational variables.

Table 2. Parameter estimates obtained from the neural network

Predictor		Predicted		
		Hidden Layer 1		Output Layer
		H(1:1)	H(1:2)	Yield
Input Layer	(Bias)	0.971	2.673	
	Glucose	0.081	0.026	
	Incubation	3.947	-2.052	
	Temperature	-1.488	2.594	
Hidden Layer 1	(Bias)			-0.623
	H(1:1)			1.144
	H(1:2)			0.878

Analysis of Connection Weights

The connection weights obtained from the trained ANN (Table 2) provide valuable insights into the relative importance of each input variable on AA yield prediction. Among the evaluated parameters, incubation time and temperature exhibited the most significant influence on model output. Incubation time showed the highest positive weight (3.947) toward hidden neuron H(1:1) and a negative weight (–2.052) toward H(1:2), indicating its dual regulatory effect on acetic acid yield formation and highlighting its complex interaction within the network. Similarly, temperature displayed a strong positive connection weight (2.594) to H(1:2) and a moderate negative weight (–1.488) to H(1:1), confirming its nonlinear relationship with incubation time and its substantial contribution to AA biosynthesis.

In contrast, glucose concentration presented relatively smaller but consistent weights across both hidden neurons, suggesting a secondary yet supportive role in the prediction process, which can be attributed to the presence of dairy wastewater in the substrate used for AA production. Furthermore, the positive output weights from both hidden neurons to the output layer (1.144 and 0.878) indicate a synergistic contribution of these hidden nodes in enhancing the predictive accuracy of the network. This pattern demonstrates that the trained ANN effectively captured the nonlinear dependencies among process variables and their combined effects on acetic acid yield.

The network diagram (Figure 1) visually depicts the flow of information across the input, hidden, and output layers, emphasizing the dominant influence of incubation time and temperature on AA yield prediction. The thickness and direction of the connections highlight the strength of these variables within the model architecture. This observation is further corroborated by the relative importance analysis, which ranked incubation time and temperature as the two most significant predictors, while glucose concentration exerted a comparatively lower yet consistent effect on model output. These results align with the connection weight analysis (Table 2), confirming that process conditions related to time and temperature play a critical role in modulating the metabolic activity of *Gluconobacter oxydans* and, consequently, the overall AA production.

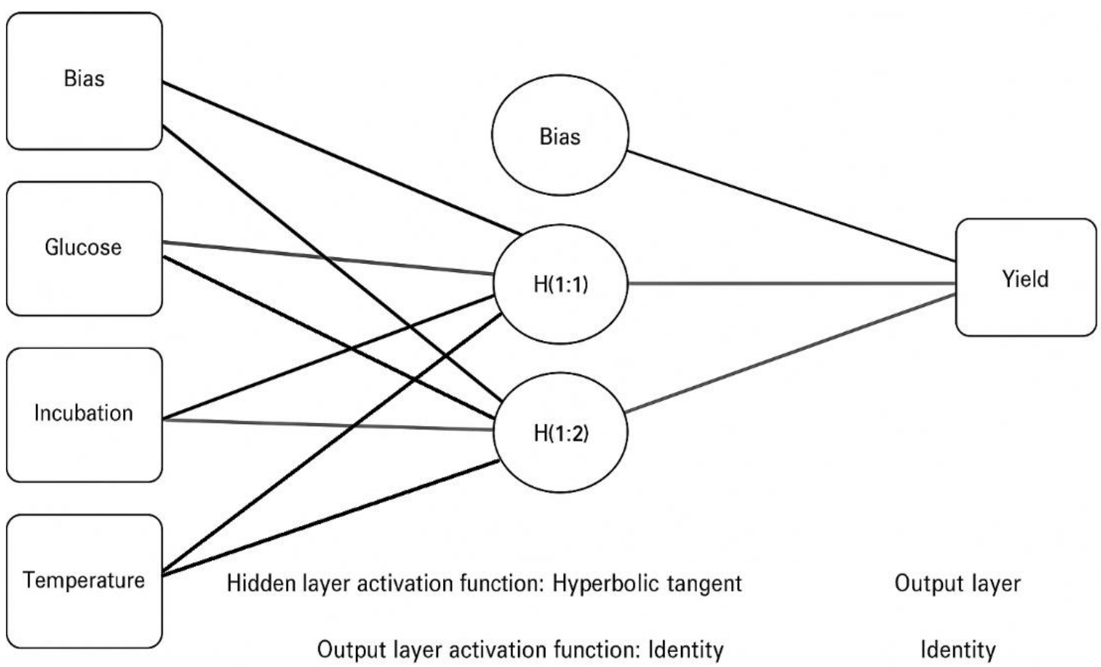

Figure 1. Schematic representation of the ANN model showing input, hidden, and output layers.

CONCLUSION

The artificial neural network developed in this study successfully predicted acetic acid yield based on the input variables of glucose concentration, incubation time, and temperature. The model exhibited low prediction errors in both the training and testing phases, confirming its robustness, reliability, and ability to capture the nonlinear dynamics characteristic of biological fermentation systems. Among the evaluated parameters, incubation time and temperature were identified as the most influential factors affecting yield, consistent with established microbial and biochemical principles, wherein metabolic activity and substrate conversion are strongly governed by process duration and environmental conditions. Overall, the developed artificial neural network demonstrates significant potential as a computational tool for process modeling, optimization, and prediction in biotechnological and food-related applications. Its effectiveness is particularly valuable in scenarios with limited experimental datasets or complex variable interactions. Future research should focus on expanding the dataset, integrating additional process parameters, and applying the trained network to simulate or optimize bioprocess performance under a broader range of operational conditions.

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