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A Neuro-Fuzzy Technique for the Modeling of β-Glucosidase Activity from *Agaricus bisporus*

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Abstract: This paper proposes a neuro-fuzzy system to model β-glucosidase activity based on the reaction's pH level and temperature. The developed fuzzy inference system includes two input variables (pH level and temperature) and one output (enzyme activity). The multi-input fuzzy inference system was developed in two stages: first, developing a single input-single output fuzzy inference system for each input variable (pH, temperature) separately, using the robust adaptive network-based fuzzy inference system (ANFIS) approach. The neural network learning techniques were used to tune the membership functions based on previously published experimental data for β-glucosidase. Second, each input's optimized membership functions from the ANFIS technique were embedded in a new fuzzy inference system to simultaneously encompass the impact of temperature and pH level on the activity of β-glucosidase. The required base rules for the developed fuzzy inference system were created to describe the antecedent (pH and temperature) implication to the consequent (enzyme activity), using the singleton Sugeno fuzzy inference technique. The simulation results from the developed models achieved high accuracy. The neuro-fuzzy approach performed very well in predicting β-glucosidase activity through comparative analysis. The proposed approach may be used to predict enzyme kinetics for several nonlinear biosynthetic processes.

Keywords: *Agaricus bisporus*; β-glucosidase; fuzzy logic; neural network; enzyme; kinetic modeling

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1. Introduction

The catalytic enzyme β-glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21) hydrolyzes the glycosidic bonds in carbohydrates to non-reducing terminal glycosyl residues, oligosaccharides, and glycosides. The β-glucosidase enzyme exists in a multitude of organisms ranging from bacteria, archaea to eukaryotes [1]. These enzymes are responsible for the conversion of biomass in microorganisms, the breakdown of glycolipids and lignification processes, the activation of phytohormones, catabolism of cell walls in plants, and the interactions between plants and microbes [2]. β-Glucosidase is a major therapeutic target for Gaucher's disease, resulting from β-glucosidase insufficiency [3]. This enzyme type is a significant component in the multi-enzyme cellulose complex and catalyzes the final step in cellulose hydrolysis. Cellulose is the most ample carbohydrate on Earth, and there is an abundance of applicable research conducted on its potential usage in numerous industries [4–7]. Cellulose enzymes catalyze cellulose to synthesize cellobiose and other short-term cello-oligosaccharides, which are hydrolyzed by β-glucosidase to glucose [5]. The dual characteristic of β-glucosidase, which allows it to synthesize as well as degrade glycosidic bonds, gives it a vast potential from an industrial point of view [2].

Current global energy demands and the increasing burden on fossil fuels have increased the need for more efficient production of biofuels at a substantial scale to replace nonrenewable fuel sources. The cellulosic biofuel production method consists of decomposing lignocellulosic biomass into sugar, followed by fermentation processes to

generate biofuel [8,9]. The complete degradation of cellulose is ultimately performed by β-glucosidase. β-Glucosidase, an enzyme that determines the rate of the total conversion of cellulose into glucose, serves as the final enzyme in lignocelluloses degradation reaction [8,10]. β-Glucosidase enzymes are also employed in the flavoring industry of bitter substances during juice extraction and aroma release from Muscat wine and apricot grape juice. Immobilized β-glucosidase particles were found to be highly stable in wine and fruit juice conditions and can be effectively used multiple times for multiple rounds of hydrolases of bound aroma [11]. Additionally, β-glucosidase has enormous potential for food processing industry applications; it is utilized as a flavoring-enhancement enzyme, can reduce the viscosity of juice, and change the color of juice products as well as other physiochemical properties [12].

Cell metabolism is a complicated dynamic system that can exhibit a wide range of dynamical events, including several steady states and temporal oscillations. Kinetic models are essential to comprehend and predict the behavior of metabolic networks quantitatively. Despite their many apparent benefits, comprehensive and thermodynamically viable metabolic models are inherently challenging to formulate and fit. They consist of a multitude of heterogeneous parameters, involve nonlinear relations, and have many convoluted interactions. Jurado et al. [13] present a mechanistic modeling approach for two commercial β-galactosidases (Lactozym and Maxilact). Ref. [13] used two kinetic models to investigate the effect of pH on enzyme activity and thermal deactivation at various ionic concentrations. In addition, the proposed models were applied to the activity versus pH experimental data collected from other researchers using free and immobilized β-galactosidases. Both models fit the experimental results acquired in the original study and the results obtained by other researchers satisfactorily. While progress has been made in the development of mechanistic kinetic models that describe specific metabolic pathways, such as the central carbon metabolism in red blood cells [14] and *E. coli* [15], glycolysis in yeast [16], the activity of β-galactosidases as influenced by pH, ionic concentration, and temperature [13], all this progress has been hampered by limitations and common problems. The complex identification of highly-parameterized models, the intrinsically abstruse nonlinear nature of mechanistic rate laws, and the utilization of in vitro kinetic data to fit and approximate enzyme kinetics in vivo are just a few of these obstacles [17]. Creating an ideal model requires the direct global fitting of detailed mechanistic models of metabolic pathways, using in vivo data. Unfortunately, strong parameter coupling and homeostatic control render such tasks impossible for extensive pathways. Simplified kinetics have been utilized broadly to bypass this limitation. Formalisms, such as log–ln kinetics [18] and generalized mass action [19], have been used to investigate the dynamic behavior of metabolic pathways, depending on the scope, application, and specific model features. While integrating formalisms with kinetic modeling frameworks resulted in invaluable insights into the metabolic network's operation [20], they offer a limited prediction power, due to their lack of kinetic details. Allosteric regulation and other complex kinetic features are essential to explain the complex metabolic behaviors in vivo [17]. Hence, the construction of detailed and accurate kinetic models from in vivo data remains a challenging task [21].

Optimum pH and temperature determination and better control of kinetic function parameters of β-glucosidase are essential for its maintained use and the commercial viability of its biosynthetic processes. Enzymatic activity in vivo involves a multi-variable process with time-varying, nonlinear, and stochastic characters [22]. Therefore, modeling metabolic pathway dynamics compels accurate and detailed kinetic formulas at the enzyme level. Precisely, kinetic formulas must account for all metabolic effectors that vastly impact in vivo pathway regulation. However, due to the issues and limitations discussed earlier, there is currently a lack of precision in kinetic information on the action of β-glucosidases. The absence of optimal kinetic models is due to a lack of mathematical tools and model discrimination approaches, such as the linearization of nonlinear models [22]. Hong et al. (1981) [23] employed an initial rate approach and Lineweaver–

Burk linear regression. However, the model could not fit the experimental data accurately, especially after long experimental times [23]. Bravo et al. (2001) [24] utilized the former model to fit the experimental data obtained in the enzymatic hydrolysis of cellobiase by β-glucosidase [24]. Several initial pH values were tested, and very good fits were achieved. However, the authors could not establish a relationship between pH and substrate and/or product inhibition. In addition, they could not establish a complete model able to duplicate the system kinetics for various pH values. Furthermore, as the majority of kinetic laws in the literature do not account for all factors simultaneously, a considerable portion of kinetic information exists in a qualitative or semi-quantitative form [25].

New theories and computing advancements have recently progressed rapidly, such as artificial intelligence control, fuzzy logic, artificial neural networks, and many others. Nonlinear models, such as artificial neural networks (ANN) and fuzzy logic, provide significant benefits over traditional mechanistic modeling, such as the ability to handle enormous amounts of noisy data from nonlinear and dynamic system processes [26]. Without exact prior information, ANN and fuzzy logic can relate input and output parameters [27]. Such novel approaches possess some new features that traditional methods lack and have an enhanced ability to solve complex problems, uncertainties, and higher standard specifications for modern industries [28]. For example, Furlong et al. proposed a fuzzy logic approach that accurately predict the enzymatic saccharification of sugarcane bagasse process behavior when the feeding policy changes. Simpler models, such as those based on Michaelis–Menten kinetics (MMK), can correspond well to the data for a particular feeding procedure. However, the same model could not accurately predict the process behavior when the feeding procedure was changed [29].

Soft computing methods, such as fuzzy logic and neural networks, are tools used to establish intelligent systems [30]. Fuzzy-neural networks combine fuzzy logic and neural networks to compound the advantages of fuzzy logic and neural networks. This combination of fuzzy-neural networks can deal with fuzzy information while also conducting fuzzy reasoning. Moreover, neural networks introduce a learning mechanism to improve the adaptive competence of the network. Hence, all of this allows the neuro-fuzzy system to simultaneously obtain reasoning capability and adaptive ability [31,32]. ANFIS is a hybrid of artificial neural networks and fuzzy inference systems (FIS). Because it relies on gradient descent and least square algorithms, ANFIS can improve any modeling system's fault tolerance, speed, and adaptability [33]. As a result, such modeling techniques are useful and versatile in modeling and estimating nonlinear systems [34]. Sreekumar et al. (2020) [35] provide a robust neuro-fuzzy inference system model derived from real plant data for predicting the pH level of sodium chlorate cells. The developed model relies on fewer measurable parameters for modeling, such as HCl, brine, and NaOH flow rates, the cell electrolyte temperature, DC load current, and feed input pH level [35].

In the present work, we briefly explain a generalized model of neural-fuzzy networks representing the effects of temperature and pH level (simultaneously) on β-glucosidase activity, extracted from *Agaricus Bisporus* (white button mushroom).The developed approach will enhance kinetic modeling, improving the kinetic control and efficiency of this industrially important enzyme.

2. Neuro-Fuzzy Inference System

Several artificial intelligence techniques were developed and investigated over the past decades, such as neural networks, evolutionary algorithms, fuzzy logic, and expert systems. Each intelligent technique has specific computational properties (such as the ability to learn and ability to interpret decisions) that make the techniques more suitable for a specific problem. Fuzzy logic systems provide an inference framework that can mimic human logical reasoning capabilities based on knowledge-based systems. Fuzzy logic is a theory that presents a mathematical approach to capturing uncertainties associated with human analytical processes [32,36]. The mapping process from an input variable to output using the fuzzy logic approach is called fuzzy inference. A basis is provided by mapping

between input and output, allowing decisions and patterns to be made or recognized. Fuzzy inference involves the following [37]:

- The process of determining the degree to which the input variable belongs to each of the suitable fuzzy sets through membership functions, also known as the fuzzification process. The membership function (MF) designates a mapped membership value between 0 and 1 for each point (input value). This method creates fuzzy sets.
- Application of the fuzzy operator in the antecedent using logical operations (AND = min, OR = max, and NOT = additive complement).
- The implication from the antecedent to the consequent using if–then rules, where fuzzy sets and fuzzy operators are the subjects and verbs of fuzzy logic. Every rule has conjugated a weight that is applied to the value given by the antecedent. The weight value is within the range [0–1].
- The aggregation process of the consequent across the rules. The fuzzy sets representing the strength of each rule's output are amalgamated into a single fuzzy set in a process called aggregation.
- The defuzzification process can be used to obtain a single output value from the output set using one of the following methods: centroid method, bisector method, middle of maxima method, largest of maximum, and smallest of maxima method.

Figure 1 shows the steps for fuzzy inference system for two inputs (X and Y) and one output (Z). The degree to which the crisp input (x1 and y1) belongs to the appropriate fuzzy sets (A1, A2, and A3; B1, B2 and B3) μ is determined in the first step. In the second step, the fuzzified inputs are applied to the antecedents of the fuzzy rules (Rule 1, Rule 2, and Rule 3) to generate the rules output. Then, the unification of the outputs of all rules is implemented in the third step. Finally, the rules output is evaluated in output fuzzy sets to produce a crisp output z1 [31].

Artificial neural systems are expressed as a basic mathematical model of brain-like systems that behave as parallel computational networks [32,36]. Most neural networks must be trained. The neural networks can capture (learn) the relation between input and outputs by updating network weights. The neural networks can adaptively learn new correlations, new functional dependencies, and new sequences and patterns. Even though neural networks are good at the recognition process, they lack result interpretation [31,32,36,38].

While fuzzy logic can directly utilize the insight of expert knowledge by using rules with linguistic labels, it is extremely time-consuming to design and tune the membership functions that delineate these linguistic labels quantitatively. Neural network learning techniques can automate this procedure, making it considerably more efficient, while improving its overall performance. The hybrid system that incorporates fuzzy logic into neural networks is referred to as a neuro-fuzzy system. Neural networks are used to adjust the membership functions of the fuzzy systems. Hybrid systems that combine fuzzy logic and neural networks is validating their effectiveness in real-world problems. The adoption of hybrid systems are expanding rapidly with much successful utilization in various applications. The employment of fuzzy logic provides an inference process under logical uncertainty and cognitive ambiguity, while computational neural networks provide beneficial and exciting advantages, such as learning, fault tolerance, adaptation, parallelism, and generalizations [32,36].

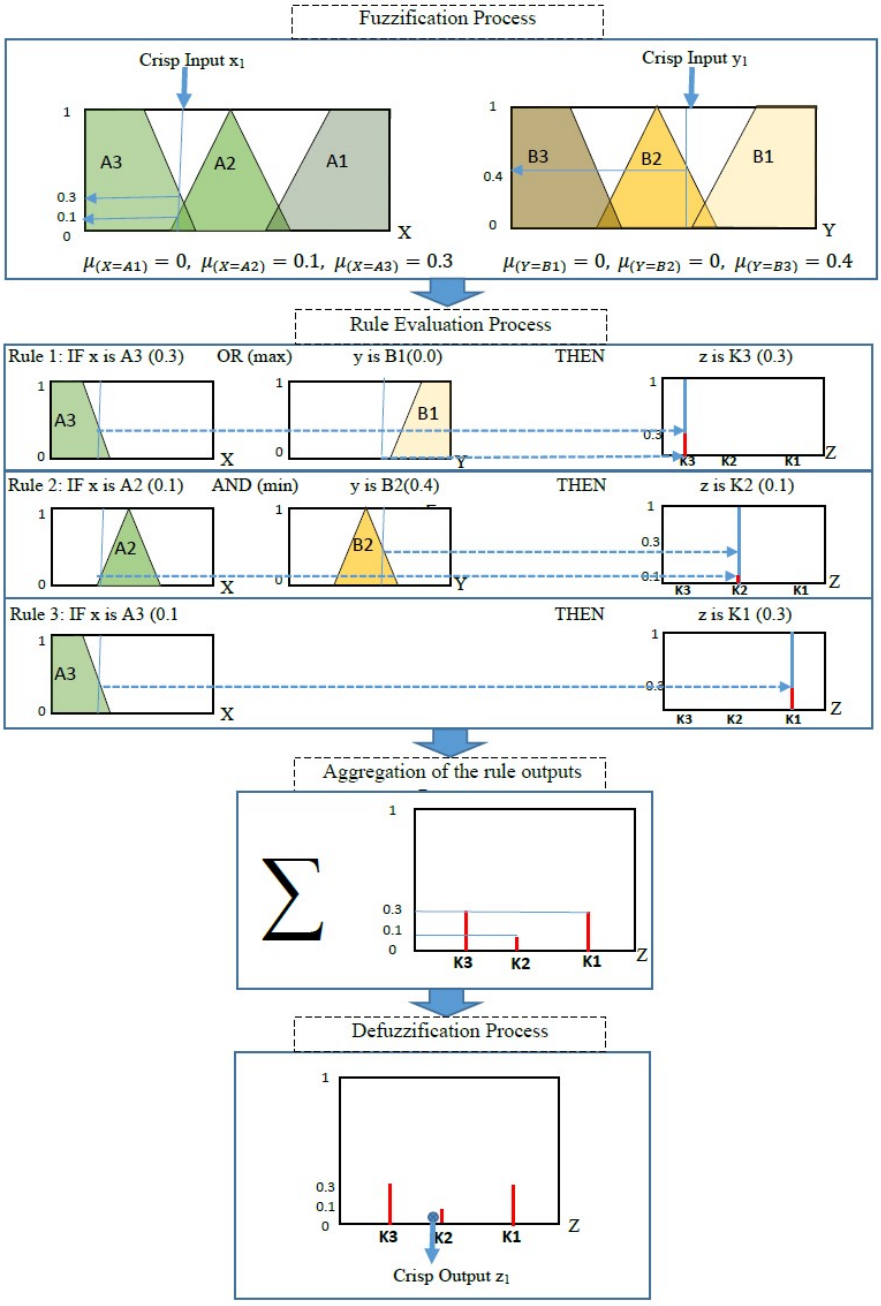


Figure 1. The structure of Sugeno fuzzy inference.

3. Enzyme Activity Modeling Using ANFIS

3.1. Sugeno Fuzzy Inference Systems

The Sugeno fuzzy inference technique, also known as Takagi–Sugeno–Kang fuzzy inference, employs singleton output membership functions based on the weighted average or weighted sum of several data points rather than computing a centroid of a two-dimensional area in the Mamdani system. In addition, the Sugeno system's membership output is either a constant or linear function of the input values, making the defuzzification method more computationally efficient [37].

For the suggested MISO FIS, each rule in the system operates as follows. Each rule generates two values, as shown in Equations (1) and (2):

$$z_i = a_i * Temp + b_i * pH + c_i \quad (1)$$

where z_i is the rule output level, which is either a constant value or a linearized function of the input values. $Temp$ and pH are the input values, and a_i, b_i, and c_i are constant coefficients. z_i is a constant ($a = b = 0$) for a zero-order Sugeno system.

$$w_i = And\ Method(F_1(Temp), F_2(pH)) \tag{2}$$

The rule antecedent determines w_i, which is the rule firing strength. Here, F_1 (...) and F_2 (...) are the membership functions for $Temp$ and pH, respectively.

The weighted output level (Enzyme Activity level), which is the product of w_i and z_i, is the output of each rule.

$$Enzyme\ Activity = \frac{\sum_{i=1}^{N} w_i * z_i}{\sum_{i=1}^{N} w_i} \tag{3}$$

where N is the number of rules.

The process of tuning a Sugeno-type fuzzy inference system using training data was developed in the 1990s [39]. The system design and learning algorithm are called the "adaptive-network-based fuzzy inference system (ANFIS)", where a fuzzy inference system is developed in the framework of adaptive networks. An input–output relation is constructed using a hybrid learning algorithm based on human insight (in fuzzy hypothetical rules) and specified input–output data.

For the current β-glucosidase modeling problem, discerning membership function parameters by inspecting data can be difficult. The input/output data will determine the membership function parameters rather than selecting the parameters via try and error or guessing. In this work, a neuro-fuzzy designer application in Matlab ® is used to create a neuro-fuzzy inference system (ANFIS) based on input/output data. ANFIS can model a system with N inputs and one output when sufficient training data are available. Sufficient training data must describe the entire problem domain. In general, training data should accurately reflect the characteristics of the data that the FIS is expected to model [37]. For this study, however, the first set of training data was extracted from the pH-enzyme activity at one temperature setting (37 °C), while the second set was obtained from the temp–enzyme activity at only one setting pH value (5.5). Hence, the available initial data points do not fully describe the entire features of the MISO output system (pH–temperature–enzyme activity). Using an inadequate training data set can lead to insufficient training for the FIS and the inability to capture and infer the relation between system inputs and output. Therefore, a new procedure was implemented to satisfactorily utilize the multi-input single-output (MISO) process for the current study. The first stage uses ANFIS to generate a SISO model of the relation between pH and β-glucosidase activity and temperature and β-glucosidase activity independently. Then, the developed SISO models' membership functions are used to generate the fuzzy inference system (FIS) for the MISO process in the second stage.

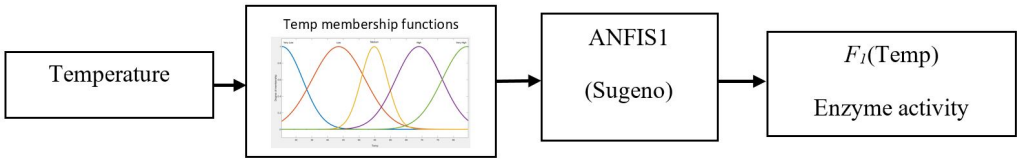
3.2. Training Data

Input/output training data were generated from the experimental results given in Ref [1]. Ašić et al. (2015) [1] conducted enzyme kinetic studies, using the substrates 4-Nitrophenyl beta-D-glucopyranoside (*p-NPGlu*) and 2-Nitrophenyl beta-D-glucopyranoside (*p-NPGlu*). Both substrates were incubated at 37 °C for 30 min in concentrations ranging from 0.36 mM to 2.5 mM in 50 mM sodium acetate buffer (pH 5.5). Ref [1] showed that *(p-NPGlu)* was the best suitable substrate for mushroom β-glucosidase. Hence, only *(p-NPGlu)* was considered for optimum pH and temperature determination in this study. For optimum pH determination, (*p-NPGlu*) and enzyme were incubated for 30 min at 37 °C in three different buffers: sodium acetate buffer (pH 3.0–6.0), phosphate buffer (pH 6.0–8.5), and glycine–NaOH buffer (pH 8.5–12.0). Then, enzyme activity was measured spectrophotometrically at 405 nM. For optimal temperature determination, the enzyme was incubated with 5 mM (*o-NPGlu*) solution under seven distinct temperatures, ranging

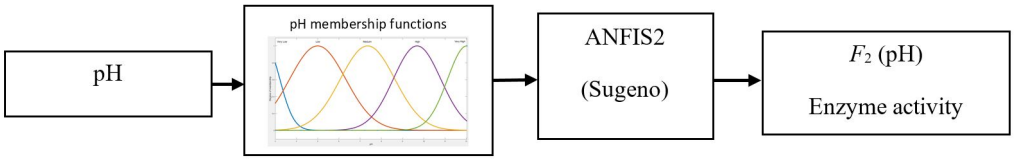
from 25 °C to 85 °C. The training input/output data were distributed consistently along with each graph to accurately represent the attributions and characteristics of the data that the trained fuzzy inference system is intended to simulate. Sixty-six data points from each graph were collected and used in this work. Both ANFIS and neuro-fuzzy designer allowed the adjustment of the optimization method, modification of the number of training epochs, and adjustment of the training error goals.

3.3. ANFIS Models for pH–Enzyme Activity and Temp–Enzyme Activity

A SISO ANFIS model is developed based on the published Mathlab® code in Ref [40] to model pH–enzyme activity and temperature–enzyme activity individually, as shown in Figure 2a,b. Developing an effective and efficient ANFIS system requires adjusting and tuning the system parameters to obtain the best performance possible. The benchmark study Figure 3 served to provide an initial estimation for the necessary membership functions (MFs) required for each SISO FIS (5 MFs for pH and 6 MFs for temperature). Furthermore, the performance of the MISO system must be tuned carefully, and several trials might be needed to achieve the best results. After several iterations, the best results were achieved using 5 MFs for both pH and temperature in the MISO model.



(**a**)- Temp- Enzyme activity FIS structure



(**b**)- pH- Enzyme activity FIS structure

Figure 2. SISO FIS models: (**a**) Temp- Enzyme activity FIS structure. (**b**) pH- Enzyme activity FIS structure.

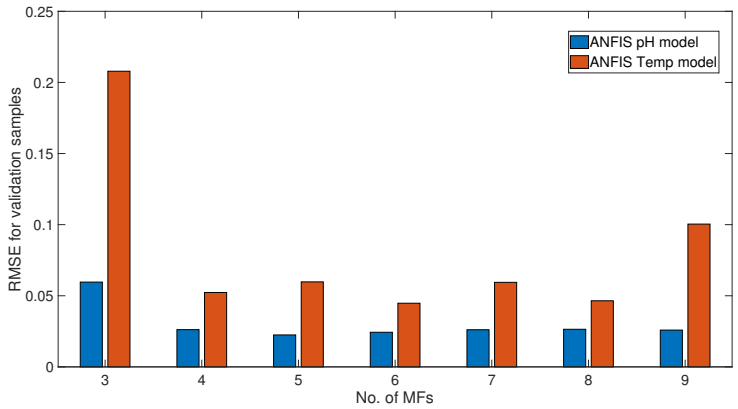


Figure 3. Effect of the number of memberships on the ANFIS models' accuracy.

The optimization method used to classify the memberships for both models is hybrid. The number of training epochs is 200, with a training error goal of 1.0×10^{-5}. After completing the training and optimization sessions for each ANFIS model, the type and parameters for input and output memberships were created, as shown in Figure 4.

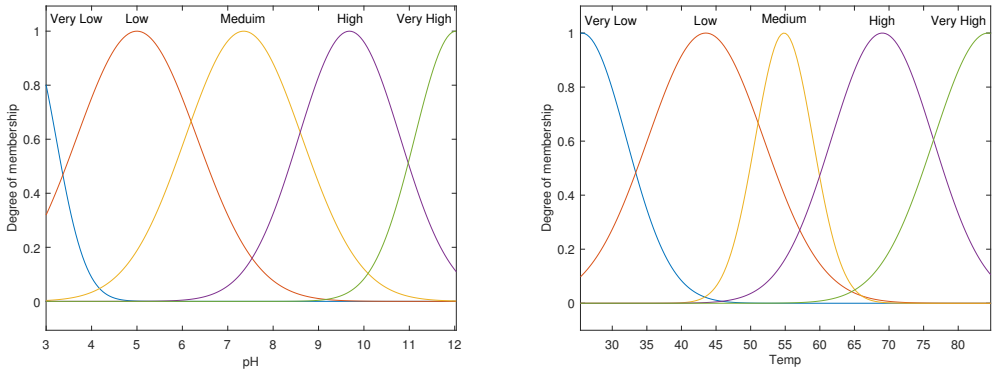


Figure 4. Memberships for pH and temperature.

4. FIS Model for pH–Temp–Enzyme Activity

A new fuzzy inference system model needs to be developed to include more than one input variable simultaneously, as shown in Figure 5. The input memberships type and parameters were transferred from previously developed ANFIS models for each input variable (pH and temp).

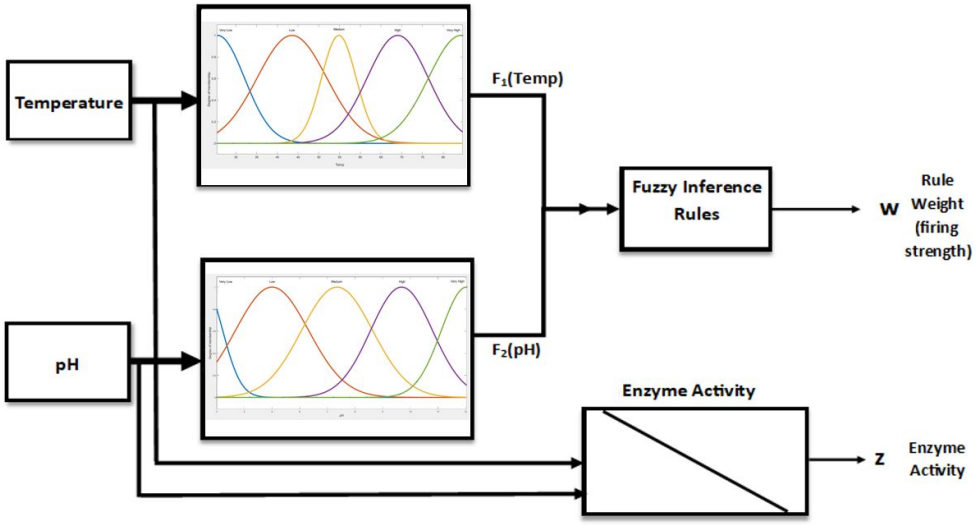


Figure 5. Multi-input single-output (pH–temp–enzyme activity) FIS structure.

A new rules base is developed to describe the expected relation between the two input variables (pH and temperature) and the output (enzyme activity) as shown in Table 1; the output surface of the developed fuzzy inference system is shown in Figure 6. The output is selected to be a singleton-constant output membership function with the following values [0.12 0.5 1.25 2.25 2.75].

Table 1. Base rule output (enzyme activity: 1 = Very Low, 2 = Low, 3 = Medium, 4 = High, 5 = Very High).

Temp	pH →	Very Low	Low	Medium	High	Very High
Very Low		2	3	1	1	1
Low		3	4	2	2	1
Medium		4	5	2	2	2
High		3	4	2	1	1
Very High		2	3	1	1	1

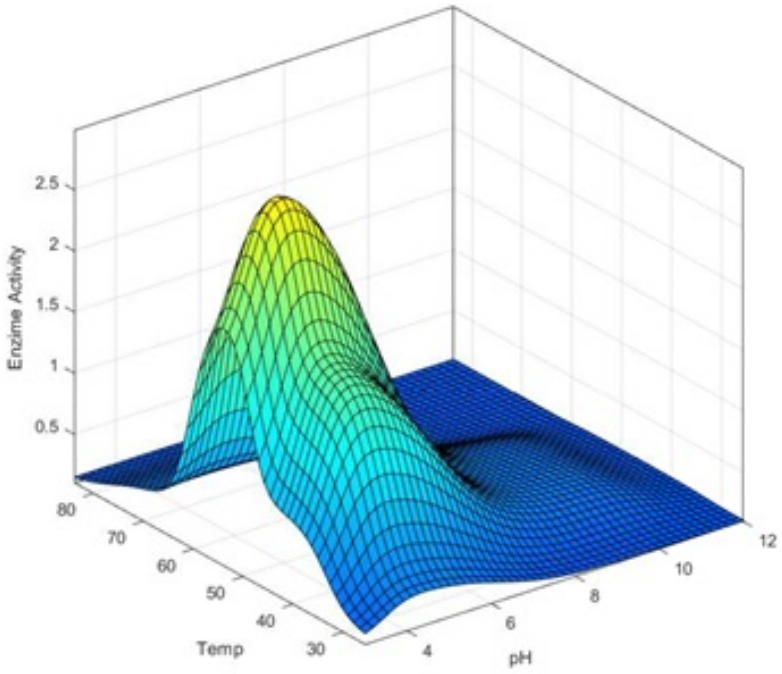


Figure 6. FIS output surface.

5. Results and Discussions

The ANFIS SISO model for the temp–enzyme activity is trained to capture the nonlinear relation between the reaction temperature and enzyme activity, as shown in Figure 7. The training goal was reached with a mean error of 6.13×10^{-9}. The validity of the SISO temp–enzyme activity model and pH–enzyme activity model is tested, using a randomly selected new data sample (15% of the original data set). Figure 8 presents the regression model between the predicted and target values for the training and testing data sets. Both training and testing sessions display good performance with an R-value of more than 0.997 for training and testing data.

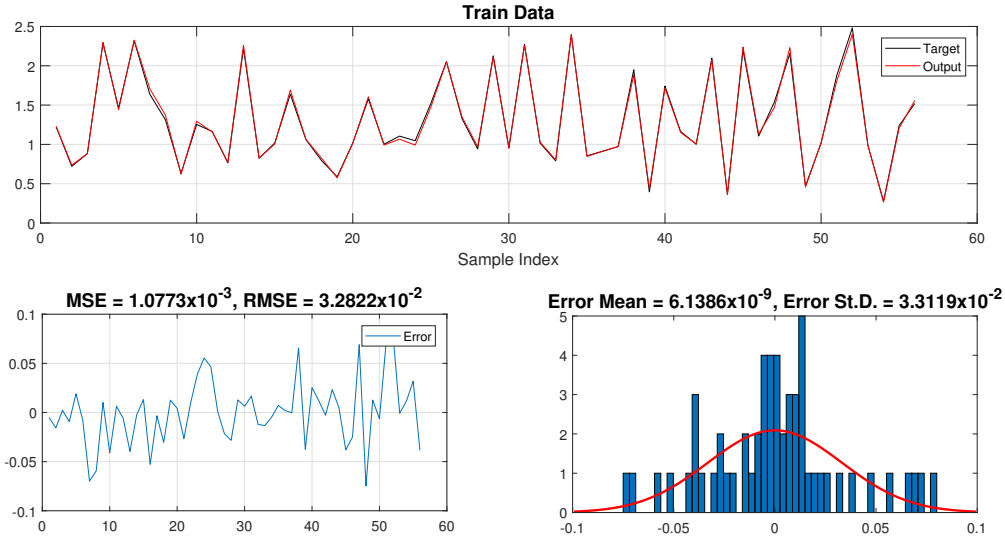


Figure 7. SISO temp–enzyme activity FIS model training.

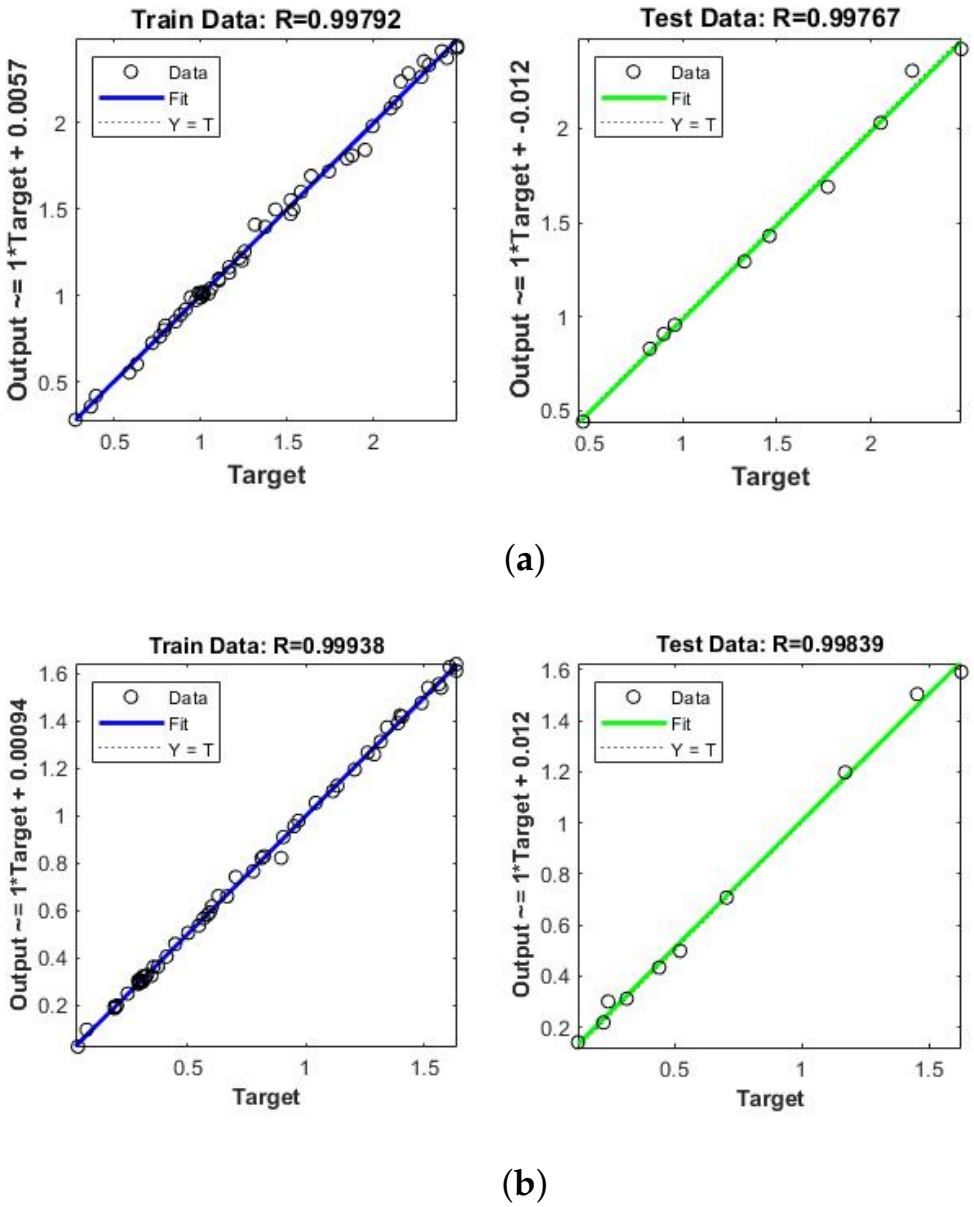


Figure 8. Validity test of SISO FIS models, using five MFs for inputs and output. (**a**) Temp–enzyme activity model performance using training data (blue) and test data (green). (**b**) pH–enzyme activity model performance using training data (blue) and test data (green).

The developed fuzzy inference systems' performance was evaluated against experimental (target) data, using different accuracy indicators. For the temp–enzyme activity model, the mean error for all data is 0.0044233, error STD is 0.038, and the RMSE is 0.0383, which means the developed FIS model successfully captured the relation between temperature and enzyme activity, as shown in Figure 9.

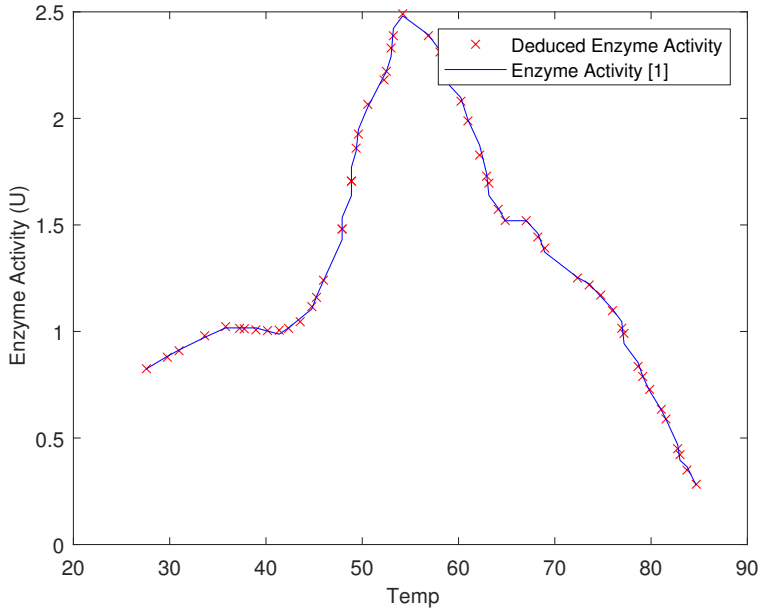


Figure 9. Performance of SISO temp–enzyme activity FIS model. (U = µmol/min), temp = °C).

The ANFIS SISO model for the pH–enzyme activity is trained to capture the nonlinear relationship between reaction pH and enzyme activity, as shown in Figure 10. The training goal was reached with a mean error of 2.577×10^{-7}.

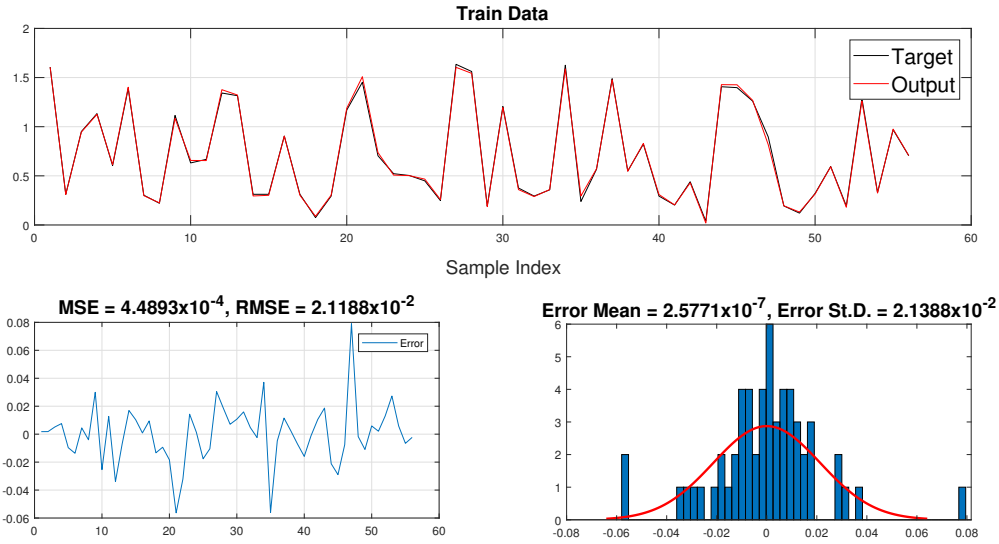


Figure 10. SISO pH–enzyme activity FIS model training.

The mean error of the pH–enzyme activity model for all data is 0.000998, Error STD is 0.0208, and the RMSE is 0.0206, indicating the developed FIS model successfully captured the relation between pH and enzyme activity, as shown in Figure 11.

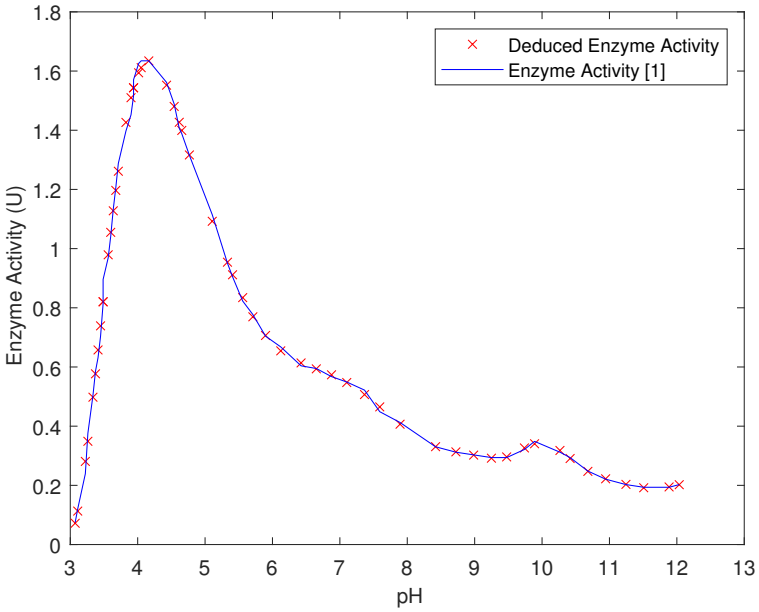


Figure 11. Performance of SISO pH–enzyme activity FIS model. (U = µmol/min).

The validity of the proposed MISO model response was investigated for two simultaneous inputs (pH and temperature), using two sets of testing points: The first group of points is set at 37 °C at varying pH values, while the second group of data points is set at a constant pH value (pH 5.5) at varying temperatures. The results are compared with equivalent points interpolated from the experimental results in Ref [1] for identical experimental conditions. The results for the selected input values are listed in Table 2.

Table 2. Performance of pH–temp–enzyme activity model performance at testing points. (U = µmol/min).

Test#	pH	Temp °C	Deduced Enzyme Activity (U) MISO Model	Enzyme Activity (U) Ref [1]	Error%
1	3.5	37	0.89	0.829	6.853933
2	4.5	37	1.48	1.51	2.027027
3	5.5	37	0.822	0.8	2.676399
4	6.5	37	0.603	0.6	0.497512
5	7.5	37	0.448	0.42	6.25
6	5.5	30	0.898	0.83	7.572383
7	5.5	40	1.0019	0.99	1.187743
8	5.5	50	2.1	2.15	2.380952
9	5.5	60	2.05	2.1	2.439024
10	5.5	70	1.4	1.29	7.857143

The simulation results show that the predicted enzyme activity using the MISO FIS model has very good matching with experimental results in Ref [1], with a mean error of 3.97% and a maximum error of 7.85%. It is also worth noting that the proposed model performed better at intermediate input values. The maximum error is recorded at the start and end limits of the provided pH and the temperature ranges, as shown in Table 2. This model response behavior can be linked to the enzyme activity's relatively low values at the selected testing points. The selected input/output membership functions and rule base for the designed MISO FIS model are more sensitive to one of the inputs (pH or temperature), which causes a slight shift to the model output from the experimental values at these points. Although the benchmark study shows that the best number of input membership functions

is five for both SISO models (pH–enzyme activity and temp–enzyme activity), as shown in Figure 6, the response accuracy based on the selected membership functions is not equal for both inputs, which affects the MISO compound model (pH–temp–enzyme activity).

6. Conclusions

A fuzzy inference system is developed to simultaneously model the effect of pH and temperature on β-glucosidase enzyme activity from *Agaricus Bisporus*.

A SISO fuzzy inference system was devised based on ANFIS techniques that combine the neural networks' learning ability to capture the nonlinear features between enzyme activity and input variables (pH or temperature) separately to tune the memberships based on the experimental results from Ref [1]. The membership parameters of the SISO systems were used to build a multi-input fuzzy inference system to include the effect of pH and temperature on the enzyme activity simultaneously. The required base rules for the developed fuzzy inference system were formulated to describe the antecedent (pH and temperature) implication to the consequent (enzyme activity) for each testing scenario. The results revealed high accuracy for the developed multi-input nonlinear model predicting the mushroom β-glucosidase activity based on specific input values (pH and temperature).

Optimum pH and temperature determination and improved control of kinetic parameters of β-glucosidase is essential for the sustained use and economic viability of its biosynthetic processes. While we used pH and temperature as our parameters for modeling kinetic activity, it is certainly feasible to use other parameters, such as salt and substrate concentration in our modeling system. Therefore, future work will address other variables involved in β-glucosidase kinetics, using the proposed modeling approach. The developed approach can be used to predict the reaction behaviors of several biosynthetic processes.

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