

Original Article

An optimized machine learning-based unit for smart arsenic electrochemical biosensors

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ABSTRACT

Biosensing platforms are analytical devices that incorporate biological receptors to detect and quantify specific analytes with high precision. Despite their effectiveness, a major limitation arises from the gradual decline in the activity and stability of immobilized bioreceptors on the electrode surface over time. This degradation necessitates frequent replacement or recalibration, thereby increasing operational costs and hindering large-scale commercialization. In the present study, a three-electrode electrochemical biosensor modified with gold (Au) nanoparticles was developed for the selective detection of arsenite, the trivalent and highly toxic form of arsenic frequently found in contaminated water resources. To improve analytical performance and predictive accuracy, machine learning algorithms were integrated into the biosensor's analytical framework. Electrochemical response data, solution pH, enzyme lifespan, and storage temperature were considered as input parameters for model development. To further enhance model efficiency, parameter optimization was performed using two metaheuristic algorithms: the firefly algorithm (FA) and the gravitational search algorithm (GSA). These were applied to tune the parameters of two predictive models: the adaptive neuro-fuzzy inference system (ANFIS) and the random forest (RF) model. Among the hybrid configurations tested, the FA-RF demonstrated superior performance, achieving a coefficient of determination (R^2) of 0.93 and a mean squared error (MSE) of 0.007. These outcomes, derived from biosensor data collected over a 45-day operational period following enzyme and Au nanoparticle immobilization, highlight the potential of metaheuristic optimization and machine learning in enhancing the reliability, sensitivity, and lifespan of intelligent biosensing systems.

1. Introduction

Arsenic is a highly toxic metalloid that occurs in the environment through both natural geological processes and anthropogenic activities such as mining, metal smelting, and the excessive use of arsenic-containing pesticides and fertilizers (Wang et al., 2013). Human exposure to arsenic—whether through contaminated water, soil, or food—poses severe health risks. Chronic exposure can lead to neurological disorders, cardiovascular diseases, gastrointestinal complications, and in extreme cases, skin lesions and cancers of the bladder, lungs, and liver (Abedin et al., 2002). These harmful effects have prompted the World Health Organization (WHO) to establish stringent guidelines for the permissible levels of arsenic in drinking water, typically set at $10 \mu\text{g L}^{-1}$, emphasizing the need for precise, real-time monitoring systems. In the natural environment, arsenic exists primarily in four oxidation states: arsenate (As^{5+}), arsenite (As^{3+}), elemental arsenic (As^0), and arsine (AsH_3) (Mascher et al., 2002). Among these, arsenite (As^{3+}) is the most mobile and toxic form, especially in groundwater systems where it predominates under reducing conditions. Its high solubility and bioavailability make it particularly hazardous, necessitating the development of analytical techniques capable of rapid, accurate, and cost-effective quantification of arsenite in aqueous media.

Over the past few decades, a wide range of methods have been explored for arsenic detection. Traditional analytical approaches include spectroscopic, chromatographic, and flow-

injection techniques (Mays and Hussam, 2009). While these methods provide high precision and sensitivity, they often suffer from several drawbacks: they require complex sample pre-treatment, involve multi-step reactions that can produce unwanted by-products, and depend on bulky, expensive instrumentation. Furthermore, such methods are generally unsuitable for on-site or point-of-care (POC) applications, as they rely on skilled personnel and laboratory conditions (Asefpour Vakilian and Massah, 2018). These limitations underscore the growing demand for portable, intelligent sensing platforms that can offer both accuracy and practicality in real-world monitoring.

To overcome these challenges, biosensors have emerged as an innovative class of analytical devices. A biosensor integrates a biological recognition element—such as an enzyme, antibody, nucleic acid, or microorganism—with a physicochemical transducer that converts biological interactions into measurable electrical or optical signals (Asefpour Vakilian, 2019; 2020). Among the various designs, electrochemical biosensors are particularly attractive due to their high sensitivity, rapid response, low cost, and ease of miniaturization. These characteristics make them excellent candidates for real-time environmental monitoring and health diagnostics. For arsenite detection, enzymatic biosensors based on the enzyme arsenite oxidase have shown significant promise. The enzyme catalyzes the oxidation of arsenite (As^{3+}) to arsenate (As^{5+}) in a redox reaction, which can be monitored electrochemically (Male et al., 2007). Such biosensors demonstrate several desirable attributes:

high specificity and selectivity toward arsenite, good reproducibility, low detection limits, and strong sensitivity under optimized conditions. Electrochemical responses are typically recorded using three-electrode systems, consisting of a working electrode, a reference electrode, and a counter electrode, with measurement outputs in the form of amperometric or voltammetric signals (Hashemi Shabankareh et al., 2023). These responses arise from redox processes or reaction inhibition events occurring at the electrode interface.

However, a major limitation of enzymatic biosensors is the gradual loss of enzyme activity over time. Once immobilized on the electrode surface, the enzyme can denature or detach due to environmental fluctuations, leading to a progressive decline in signal intensity. In many cases, the biosensor's sensitivity decreases by more than 10% within just a few days of enzyme immobilization. This deterioration necessitates frequent enzyme replacement or recalibration, increasing operational costs and complicating commercial deployment (Kalimuthu et al., 2015; Asefpour Vakilian, 2022a; 2022b). The challenge, therefore, lies in enhancing both the stability and longevity of these devices without compromising analytical accuracy.

To address these issues, researchers have begun integrating machine learning (ML) into biosensing systems, giving rise to the concept of intelligent biosensors. These devices combine traditional biochemical sensing with data-driven computational intelligence, enabling adaptive, self-correcting, and predictive performance (Massah and Asefpour Vakilian, 2019). ML algorithms analyze not only the raw electrochemical signals generated by the biosensor but also contextual and operational parameters—such as enzyme lifespan, temperature, pH, and storage conditions—to correct for signal degradation and improve measurement reliability over time. By learning from past data, intelligent biosensors can predict analyte concentrations even when sensor performance deteriorates, significantly extending their useful lifespan. A variety of ML models have been successfully applied to biosensing applications, including artificial neural networks (ANNs), support vector machines (SVMs), decision trees (DTs), random forests (RFs), Naïve Bayes classifiers, fuzzy inference systems (FIS), and Adaptive Neuro-Fuzzy Inference Systems (ANFIS) (Cui et al., 2020).

Such models can capture complex nonlinear relationships between input variables and outputs, making them particularly well-suited for electrochemical systems where multiple factors simultaneously influence sensor response. Despite their success in other analytical domains, ML-assisted biosensors for arsenic detection remain scarce. Developing such systems represents a critical step toward commercially viable arsenite biosensors that combine chemical specificity with computational adaptability, ultimately reducing the need for frequent maintenance and recalibration. However, the performance of ML models strongly depends on the tuning of their internal parameters—for example, the number of hidden neurons in a neural network or the maximum depth of a decision tree (Asefpour Vakilian, 2025). Improper parameter selection can lead to overfitting, underfitting, or unstable predictions, undermining the reliability of the biosensor. To achieve optimal results, these parameters must be fine-tuned through optimization techniques. This is where metaheuristic algorithms play a vital role.

Metaheuristic optimization methods are computational strategies designed to find near-optimal solutions to complex, multidimensional problems where traditional optimization approaches may fail or become computationally expensive (Samadi et al., 2025). Inspired by natural, physical, or social processes, they include popular algorithms such as genetic algorithms (GA), particle swarm optimization (PSO), firefly algorithm (FA), gravitational search algorithm (GSA), and grey wolf optimizer (GWO). These algorithms balance exploration (searching new regions of the solution space) and exploitation (refining known good solutions) to efficiently converge toward

high-quality results. In the context of intelligent biosensors, metaheuristics can optimize ML model parameters, improve generalization capability, and enhance prediction accuracy.

A literature review shows that although ML-based algorithms have been developed for smart arsenic biosensors, the prediction performance criteria are rather poor, especially when the lifespan of the enzyme immobilized on the working electrode of the biosensors exceeds 40 days. Moreover, the review shows that some efficient optimization methods, such as FA and GSA, have not yet been used to optimize the hyperparameters of models used in the decision-making unit of smart arsenic biosensors. It seems that utilizing such robust optimization methods can provide us with a more reliable ML-based decision-making unit in the structure of the arsenic biosensor. Therefore, in this study, we present a novel framework that integrates ML and these metaheuristic optimization into an electrochemical arsenite biosensor. The system employs a three-electrode configuration modified with gold nanoparticles (AuNPs) to enhance electron transfer and catalytic efficiency. Electrochemical data collected from arsenite-contaminated samples were combined with operational parameters such as pH, enzyme age, and storage temperature to form the input dataset for model training. The two metaheuristic algorithms—the FA and GSA—were used to fine-tune the hyperparameters of ANFIS and RF models implemented in this work, respectively.

2. Material and Methods

The proposed arsenite biosensor integrated with an ML unit is illustrated in Figure 1. In this system, the electrochemical sensing module generates response data during arsenite detection. These electrochemical outputs, combined with sample characteristics and operational parameters such as pH, enzyme age, and storage temperature, are utilized as input variables for the machine learning model.

2.1. Electrochemical unit

The enzyme arsenite oxidase, essential for catalyzing the redox reaction at the biosensor's working electrode, was extracted from *Rhizobium* sp. strain NT-26. The extraction and purification procedures were performed following the protocol established by Male et al. (2007), ensuring high enzyme activity and stability suitable for biosensing applications. For the electrochemical measurements, a point-of-care framework described by Asefpour Vakilian and Massah (2018) was adopted, providing a robust platform for rapid and reproducible bioanalytical detection of arsenite in various samples. As a standard control, chronoamperometry was employed to measure arsenite concentrations, allowing precise quantification and comparison of enzymatic activity. The biosensor was constructed using a conventional three-electrode configuration, with platinum (Pt) serving as the counter electrode, Ag/AgCl as the reference electrode, and glassy carbon (GC) as the working electrode.

To improve the electron transfer efficiency and enhance the electrochemical signal, gold nanoparticles (AuNPs) were electrodeposited onto the GC working electrode from a solution containing 0.5 M H₂SO₄ and 0.15 mM Na[AuCl₄] (Finot et al., 1999). This electrodeposition process achieved a surface density of approximately 1×10⁻⁶ g/cm² of nanoparticles, providing a highly conductive and catalytically active interface for enzyme immobilization. Following nanoparticle deposition, the enzyme was immobilized onto the electrode surface using a galvanostatic technique, applying a constant current of 10 μA for 15 minutes. This approach ensured stable attachment of arsenite oxidase while maintaining its catalytic functionality, thereby facilitating efficient electron transfer during the redox reaction and enabling accurate, sensitive detection of arsenite.

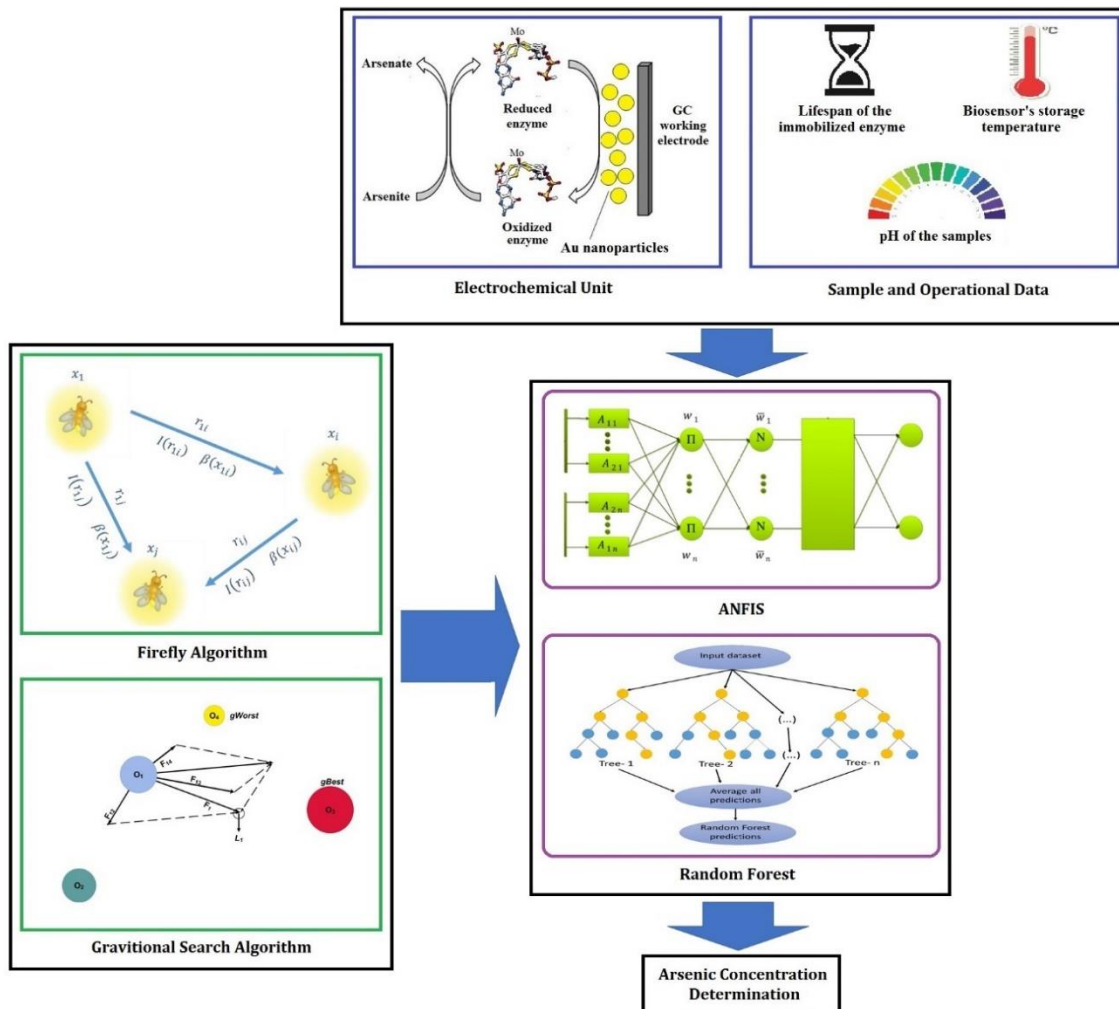


Figure 1. Schematics of the intelligent biosensor for the measurement of arsenite

2.2. Machine learning unit

We employed a MATLAB-based computational framework to implement and evaluate ANFIS and RF models for the prediction of arsenite concentrations in aqueous samples. These models were designed to incorporate multiple input parameters, including the electrochemical characteristics of the samples, the operational lifespan and storage temperature of the configured working electrode, and the pH levels of the samples under investigation. By leveraging these diverse inputs, the models aimed to accurately predict arsenite concentrations under varying experimental conditions, thereby enhancing the analytical capabilities of the proposed biosensing platform. For the ANFIS model, extensive parameter optimization was conducted to achieve an optimal predictive configuration. Specifically, we focused on tuning the number of membership functions assigned to each input, adjusting the rule weights, and refining the learning rate. This parameter optimization was carried out using advanced metaheuristic optimization algorithms, namely the FA and GSA. These algorithms were employed to systematically explore the parameter space and identify configurations that maximized the predictive performance of the arsenic biosensor. In the case of the RF model, several critical hyperparameters were similarly optimized, including the number of decision trees in the ensemble, the maximum depth of each tree, the minimum number of samples required to execute a split, and the minimum number of samples per leaf node. Fine-tuning these parameters was essential to enhance the model's ability to capture complex relationships within the data.

Prior to applying these metaheuristic optimization strategies, a comprehensive database was developed to support the training and evaluation of the machine learning models. This database was compiled from extensive measurements obtained from the electrochemical unit of the biosensor. It included data from a total of 51 standard samples of arsenite concentrations ranging from 0 to 1000 nM (with 20 nM spaces). In addition to the concentration data, the database incorporated operational and environmental features critical to biosensor performance. These features included the lifespan of the immobilized enzyme (days 1, 15, 30, 45, and 60 after the immobilization), the storage temperature of the working electrode (from 2 to 8 °C, with 2 °C spaces), and the pH values of the sampled solutions (at three levels: 6, 7, and 8). Standard samples were analyzed using the biosensor immediately following the immobilization of gold nanoparticles and arsenite oxidase, and measurements were recorded over a 60-day period. This data collection allowed for the generation of a diverse and robust dataset containing $51 \times 4 \times 3 = 612$ samples for each lifespan level.

To optimize the ML models, the parameters of FA and GSA were set according to Table 1. The selection of these parameter values involved a combination of trial-and-error experimentation and the incorporation of empirically recommended values from previous studies. During model optimization, 70% of the database samples were allocated for training purposes, while the remaining 30% were reserved for testing. The primary objective of the optimization process was to minimize the mean squared error (MSE) associated with the prediction of arsenite concentration, which served as the fitness function for both optimization algorithms.

Table 1. The parameters of the optimization methods used in this work and their values

Method	Parameter	Value
FA	Population size	50
	Maximum number of iterations	100
	Attractiveness at zero distance	1
	Light absorption coefficient	0.5
	Randomization factor	0.1
GSA	Population size	50
	Maximum number of iterations	100
	Initial gravitational constant	50
	Gravitational decay rate	20

Following the optimization of the ANFIS and RF models, their performance was rigorously evaluated using a five-fold cross-validation approach. This evaluation framework ensured a robust assessment of the models' generalizability and predictive accuracy. Performance metrics, including the MSE and the coefficient of determination (R^2), were calculated to quantify the predictive reliability of the models. While numerous error-based evaluation criteria exist for assessing machine learning models, MSE and R^2 were chosen for this study due to their effectiveness in providing a clear understanding of model behavior and predictive capability in practical arsenite detection tasks. The combination of these optimization and evaluation procedures enabled the development of a highly reliable and intelligent component within the proposed smart biosensor system.

3. Results and discussion

Previous research has consistently demonstrated the effective performance of the electrochemical component of enzyme-based arsenite biosensors, particularly when utilizing a freshly prepared working electrode that has been recently immobilized with both the enzyme and gold (Au) nanoparticles (Tabibi et al., 2022). These studies primarily focused on short-term performance, emphasizing the biosensor's sensitivity and selectivity immediately after electrode preparation. While such studies confirmed the fundamental operational capabilities of the biosensor, they generally did not examine the long-term effects of enzyme stability or how variations in operational and sample-specific factors influence biosensor behavior over time. This limitation represents a significant knowledge gap and served as the primary motivation for the present investigation.

To address this gap, the current study incorporated a decision-making unit into the biosensor, effectively transforming the conventional biosensor into an intelligent or smart biosensor capable of adapting to temporal variations in electrode performance. This approach allowed for the reliable prediction of arsenite concentrations several weeks after the initial immobilization of both the enzyme and Au nanoparticles onto the working electrode. The inclusion of Au nanoparticles is particularly important due to their unique physicochemical properties. Their high surface-to-volume ratio increases the effective surface area of the electrode, which facilitates electron transfer between the immobilized enzyme and the electrode surface. This enhanced electron transfer improves both the sensitivity and response speed of the biosensor. Additionally, Au nanoparticles stabilize the enzyme immobilization, preventing leaching and denaturation over time, and thereby extending the functional lifespan of the biosensor (Jiang et al., 2018).

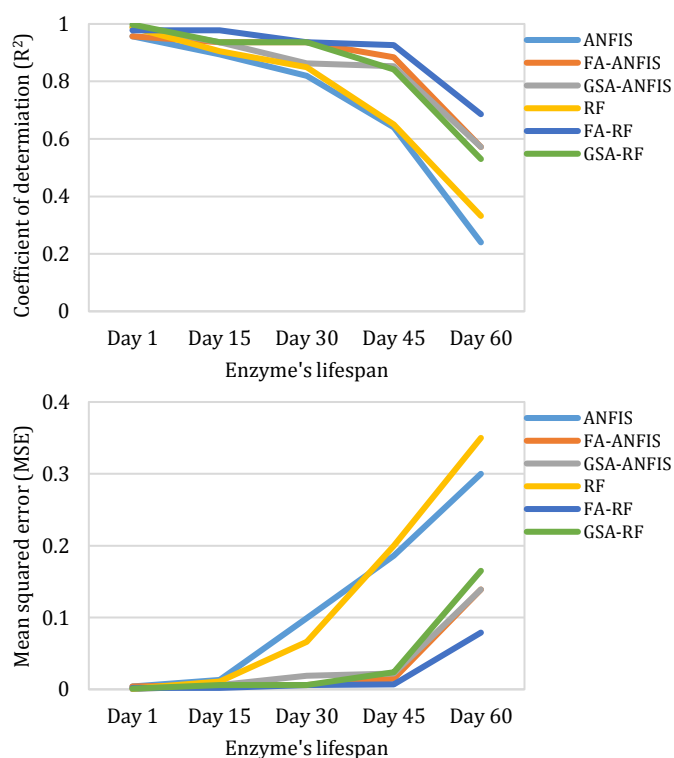
Table 2 shows the values of ML model hyperparameters found by the optimization methods used in this work for reliable prediction of arsenite using the smart electrochemical biosensor. The predictive performance of various machine learning algorithms, optimized using metaheuristic optimization techniques, was evaluated for standard samples with arsenite concentrations ranging from 0 to 1000 nM. According to Figure 2, the hybrid FA-RF model achieved an R^2 of 0.93, maintaining high predictive accuracy even 45 days after the immobilization of Au

nanoparticles and arsenite oxidase on the working electrode. This performance underscores the effectiveness of combining optimized machine learning models with a structurally enhanced biosensor to achieve long-term reliability. Importantly, the base machine learning models, when employed without optimization, exhibited considerably lower predictive performance.

The ANFIS model, without parameter optimization demonstrated limited accuracy in predicting arsenite concentrations. Similarly, RF with default tree functions and unoptimized parameter values was unable to provide reliable predictions even within the first two weeks following electrode preparation. These findings highlight the crucial role of optimization in enabling machine learning models to capture complex, non-linear relationships between electrochemical signals and sample properties. The mechanistic role of Au nanoparticles further complements this intelligent framework. By improving electron transfer rates and stabilizing enzyme immobilization, Au nanoparticles reduce noise in electrochemical signals, thereby providing cleaner input data for the machine learning models. The combination of enhanced signal quality and optimized predictive algorithms allows the smart biosensor to maintain high performance over extended periods, even as the enzyme's activity gradually declines or operational conditions vary.

Table 2. The values of ML model hyperparameters found by the optimization methods

Model	Hyperparameter	Optimal value found by optimization methods	
		FA	GSA
ANFIS	Number of membership functions assigned to each input	[5,3,2]	[5,2,2]
	Rule weights	[0.4-1.1]	[0.7-2.5]
	Learning rate	0.7	0.3
RF	Number of decision trees in the ensemble	8	2
	Maximum depth of trees	3	3
	Minimum number of samples required to execute a split	6	5
	Minimum number of samples per leaf node	3	4

**Figure 2.** Performance of predicting the arsenite concentration of samples analyzed by the smart biosensor developed in this study

The implications of these findings are significant for the development of intelligent biosensors designed for real-world applications. Integrating machine learning models as decision-making units improves predictive performance, extends operational lifespan, and enhances the suitability of biosensors for field-based or point-of-care testing (Asefpour Vakilian, 2021). This approach addresses a critical limitation in the biosensor field, namely the challenge of miniaturization, commercialization, and long-term reliability. By incorporating machine learning optimization, biosensors can adapt to changes in enzyme activity, electrode condition, and sample properties, thereby achieving a combination of durability, accuracy, and commercial feasibility (Zhang et al., 2021). In total, this study demonstrates that the integration of advanced machine learning algorithms, optimized via metaheuristic techniques, with Au nanoparticle-enhanced biosensor platforms significantly improves both predictive accuracy and long-term stability. The FA-RF model, in particular, emerged as the most effective decision-making framework for the smart arsenite biosensor, outperforming ANFIS due to its robust tree-based architecture and optimal hyperparameter selection.

The RF outperformed the ANFIS probably because it generalizes better on complex, highly nonlinear, or noisy data. RF's ensemble of decision trees scales its capacity easily, handles mixed data types, and avoids overfitting through bootstrap aggregation and random feature selection (Samadi et al., 2025), whereas ANFIS relies on a fixed fuzzy rule base that can explode or misrepresent the true mapping. ANFIS assumes smoothness and is sensitive to rule number, membership functions, and initialization, making it fragile. In contrast, RF naturally models piecewise or discontinuous functions, and is robust to noise, making it more practical for messy real-world problems.

Table 3 presents a comparative overview of selected smart biosensing frameworks that incorporate machine learning techniques for the detection and quantification of ions in the environment. Within this domain, significant research attention has been directed toward the detection of nitrate, given its widespread prevalence as a key environmental pollutant. As highlighted in the table, the majority of previous studies have relied on electrochemical biosensing components, often in conjunction with mediators such as viologen derivatives, to facilitate efficient electron transfer between the immobilized enzyme and the working electrode. The inclusion of such mediators has been shown to enhance the sensitivity and accuracy of the biosensors, which is reflected in the high R^2 values reported in these studies.

Table 3. Comparison of some smart electrochemical biosensors equipped with machine learning methods for the measurement of ions in samples

Analyte	Biosensing data	Machine learning method	R^2	Reference
Nitrate	Electrochemical data using a mediator	SVM	0.93	Massah and Asefpour Vakilian (2019)
		GA-ANN	0.97	Asefpour Vakilian (2022a)
		PSO-ANN	0.97	
		GA-SVM	0.91	Asefpour Vakilian (2022b)
		WOA-ANN	0.93	
Arsenite	Electrochemical data using Au nanoparticles	WOA-ANN	0.82	Asefpour Vakilian (2023)
		WOA-SVM	0.81	
		HHO-ANN	0.85	
		HHO-SVM	0.89	
		FA-ANFIS	0.88	This study
		GSA-ANFIS	0.85	
		FA-RF	0.93	
GSA-RF	0.84			

These findings underscore the effectiveness of mediator-assisted electrochemical transduction in providing precise measurements of target analytes. A smart biosensing platform was developed in previous studies (Asefpour Vakilian, 2023) to predict arsenite in samples by having the lifespan of the immobilized enzyme as well as sample features. The models used in that work included ANN and SVM, reaching a maximum R^2 of 0.89 after 45 days of electrode preparation. However, the model introduced in this work performed better to create a path for future studies on portable arsenite biosensors. By integrating sophisticated machine learning models, this study provides a predictive framework that can efficiently process biosensor outputs to accurately determine analyte concentrations, thereby extending the practical applicability of the device for real-world monitoring.

This approach is particularly beneficial for operators, chemists, and environmental scientists engaged in the assessment of water quality in surface water and groundwater resources, where rapid and reliable detection of pollutants is critical. Notably, the electrochemical component of the biosensor developed in this work is designed to generate minimal voltammetric data, which significantly reduces computational complexity while maintaining accurate measurement capabilities. Unlike approaches that rely on fuzzy inference systems or similar expert-driven methodologies requiring manual definition of membership functions (Esmaili et al., 2021), the machine learning methods employed here are readily implementable by engineers and practitioners without specialized expertise in fuzzy logic. This design consideration enhances the accessibility of the technology for the development of point-of-care monitoring devices, facilitating faster deployment and broader adoption in environmental and industrial contexts (Javidan et al., 2023).

4. Conclusion

This study presents an intelligent biosensor for the detection of arsenite. Among the evaluated models, both ANFIS and RF demonstrated superior predictive performance, while the FA effectively optimized model parameters. The integrated system maintained high efficiency up to 45 days following electrode preparation, highlighting its long-term stability. By combining robust electrochemical sensing with optimized machine learning, the proposed biosensor offers a practical and scalable solution for arsenite monitoring, providing a significant step toward the development and commercialization of intelligent, field-deployable biosensors. Although biosensors are now costly due to the high costs of electrodes and enzyme materials, the specificity and working range offered by arsenic biosensors are favorable compared to conventional techniques in agricultural monitoring. Moreover, future studies on developing screen-printed carbon electrodes and synthetic enzymes can help reduce the costs in the biosensor industry remarkably.

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Competing interests

No competing financial interests or personal relationships are known to the authors that could have influenced this study.

Data availability statement

The data supporting the results of this study are available from the corresponding author upon reasonable request.

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