

A Novel Approach to Detect Sulphate-Reducing Bacteria - Main Contributor of Microbiologically Influenced Corrosion

Earn Tzeh Tan School of Electrical and Electronic Engineering, Universiti Sains Malaysia, Penang, Malaysia

Zaini Abdul Halim School of Electrical and Electronic Engineering, Universiti Sains Malaysia, Penang, Malaysia

Keywords

Sulphate-Reducing Bacteria, Corrosion, Embedded System, Artificial Neural Network

S ulphate-reducing bacteria (SRB) is an anaerobic microorganism that has long been identified as one of the main contributor to the pipeline corrosion problem experienced in gas, petroleum, and water industry. The corrosion issue causes billions of dollars worth of damage each year and may lead to the deterioration of the quality of oil and water under the corroded pipeline. Currently, there are few kits and techniques available in the market targeted for early detection of SRB. Nevertheless, those detection methods have some crucial drawbacks, such as long detection period or have difficulty to conduct field test. Thus, this article proposes a rapid, accurate, and portable embedded-based electronic system to detect the presence of SRB. Preliminary experiment was conducted in lab to evaluate the function and the capability of the system. Based on the findings, the proposed technique was proven to be able to identify whether SRB presence in a presented sample within 1 hour. In addition, the system also includes data logging functionality to help users monitor the growth of SRB from time to time to reduce the damage caused by the corrosion.

Introduction

In the past few decades, the occurrence of Microbiologically Influenced Corrosion (MIC) in industrial pipelines had become a critical issue. While MIC is responsible for 20% of the total cost of corrosion, the figures of the maintenance and replacement of the corroded metallic material due to the MIC is staggering. According to an article reported by World Corrosion Organization (WCO), the annual cost of corrosion worldwide is \$2.2 trillion, more than 3% of the world's gross domestic product (GDP) [1]. Among the MIC causative bacteria, the presence of Sulphate-reducing bacteria (SRB) has been recognized as the principal contributor to the corrosion take place in buried pipeline.

SRB can be found in soil, deep well, fuel, or any Sulphate-rich wastewater produced by industrial process. The enzymes generated by the SRB have strong power to accelerate the reduction of Sulphate compounds to the corrosive hydrogen sulphide (H_2S) , which leads to the severe corrosion of iron material. Besides of the pipeline corrosion, the presence of SRB in water increases the corrosiveness of the water due to the (H_2S) produced. Moreover, the occurrence of SRB in crude oil may results to the degradation of fuel quality, causing a huge disaster to the oil and gas industries.

Considering the undesired impact and damage caused by the presence of SRB, a quick detection and accurate quantification to the presence of SRB is crucial. Nowadays, there are a wide range of corrosion inhibition technologies and chemical approaches invented to tackle the presence of the SRB in water, oil, and gas production. Nevertheless, the early investigation of the information on the diversity and distribution of SRB is still very limited. Consequently, considerable efforts have been directed towards the innovation of a quick, promising, and dependable approach to detect and quantify the presence of SRB in the natural and industrial environments.

In this article, a microcontroller-based embedded system is proposed to be a rapid and highly sensitive method for screening the presence of SRB. The two major contributions of the proposed system are described as follows:

- (1) The proposed system works similar like the culture-based methods reported by other scholars. The sample containing bacteria is grown to a sufficient level until the detector able to screen the presence of SRB. However, instead of observation based on unaided human eye, an artificial neural network (ANN)-based electronic sensing system is developed. The proposed system intends to shorten the detection period from few days to 1 hour.
- (2) The developed framework of the system aims to further enhance the users experience. The testing procedure and user interface are simplified and automated as much as possible, so that the device is simple enough that everybody can do the testing properly. In addition, the system includes data logging capability so that the user can monitor the growth of the bacteria continuously and take proper action against the bacteria at the right time to decrease the risk of corrosion. The proposed embedded design framework can be used in other electronic nose applications.

The remainder of this article is organized as follows. Section 2 briefly presents some related works. Section 3 details the methodologies of the proposed system. Section 4 provides the experimental results. Finally, section 5 concludes the work done.

Previous Detection Methods & Techniques

Currently, the approaches employed to detect the presence of SRB are divided into two main categories: culture-based and molecular-based methods. For the purpose of self-completeness, the mechanisms of these two methods are briefly discussed and the works of scholars who have made contributions for the detection of SRB are reviewed.

Culture-Based Method

Typically, the investigation of SRB using culture-based approach involves the isolation and characterization of the microorganisms by using different growth media, such as nutrient agar. According to the previous reports, the isolation of the SRB requires selective growth medium and several findings claimed that there is no growth of the bacteria takes place in the media rendered "biologically free" of iron [2]. Currently, there are a wide range of different growth media had been formulated to cultivate the growth of SRB, such as API, Postgate, Starky and Baar's [3]. The slightly different in the chemical composition of each media results different efficiency on the detection of SRB growth. Typically, the detection of SRB using culturing approach is based on human observer. There will be some blackening in the sample (illustrated in Fig. 1) when the colonies of SRB are sufficiently high enough to be observed. The major drawback of this approach is the long duration of incubation and cultivation. A current finding reported that the growth of SRB isolated from the failure-shipping pipeline could only be observed and detected after 2 to 7 days of incubation [2].



Fig 1. Indication of the presence of SRB in a sample [4]

Molecular-based Approach

Recently, the investigation and analyses of the microbial populations using culture independent molecular technique catch expertise's interest. One of the common techniques used to detect and quantify SRB populations is based on the analyses of polymerase chain reaction (PCR). The common procedure using PCR approach for SRB detection involves the extraction of RNA from an environmental sample. The result generated by the PCR reflects a mixture of microbial gene signatures from all organisms presented in a sample [5]. PCR amplification of conserved genes such as 16S rRNA has been used extensively in the

analyses of SRB distribution using molecular-based approach. However, this approach required a high skill people to conduct the testing, and it is not convenient for field test. In addition, there are several problems encountered during the process, for example, the difficulties of using rRNA fluorescent probes in sediments or industrial wastewater [6].

Methodology

Early studies revealed that the two approaches practiced currently have major drawbacks. Thus, an alternative solution was proposed in this work. Here, the general ides of the proposed SRB detection system are first presented. Next, the design of the entire hardware architecture of the proposed system is described, followed by the detailed description of software module.

General Idea

The investigation of the presence of SRB initiated with the sample preparation. The testing sample used in this work was obtained from the Industrial Biotechnology Research Laboratory, School of Biological Science, Universiti Sains Malaysia (USM). The sample was prepared in a tightly sealed universal bottle. The bottle containing isolated SRB that are streak on the surface of the Difco nutrient agar, which act as the substrate medium for SRB cultivation.

Similar with the working procedure of the culture-based methods, the prepared samples were place inside an incubation chamber for an hour. The temperature level inside the incubator was maintained at 34 degree Celsius to promote the growth of the bacteria. Next, the cap of the incubated sample was unsealed and replaced with a cap that integrates with sensors. The combined signals produced by the sensors were analyzed and the pattern shows the presence of SRB is recognized using a classification model.

In this work, a gas sensor and a temperature sensor were used to investigate the changes of H_2S and temperature under the presence of SRB. These two parameters were selected since SRB produces H_2S and releases heat in their energy metabolism. Based on the pattern of these two values, the sample can be classified whether the presented sample containing SRB or not.

Hardware Architecture

The main device in the circuit design of the proposed system is a PIC microcontroller. The controller performs data acquisition from the sensors through ADC and serial channels. Once the signal of the sensors obtained, the recognition algorithm involving artificial neural network (ANN) are executed. The output generated by the ANN shows the classification results. The results obtained are displayed on OLED. In order to increase convenience of the users, the proposed system also includes a Bluetooth module that will transmit all the obtained data including sensor signals and detection results to a tablet. An application was developed in the tablet to perform the wireless remote monitoring. In addition, every data and results presented on the tablet can be saved and continuously monitoring from time to time. Fig. 2 illustrates the overall system architecture.

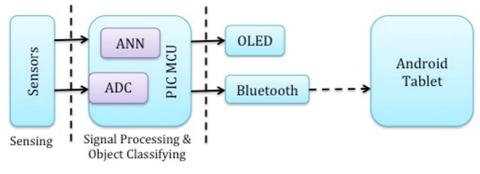


Fig 2. System Architecture

Software Module

The software controls the entire processing system in the system developed in this work so that the system can be expanded and improved without major changes in the circuit designs. The developed software mainly consists of three routines, which are data acquisitions, data transmission and ANN-based pattern recognition system. The data acquisition routine involves ADC and serial communications between the sensors and the controller, whereas the data transmission involves transmit and receive signals between the controller and the tablet through UART protocol via Bluetooth module. The ANN implemented in this work is a computation model that can classify and recognize pattern through learning. Prior to implementation, the ANN model is constructed, simulated, and trained in Matlab. The constructed ANN model is trained with back propagation algorithm to obtain the optimum value of weights and biases for each neuron presented in the ANN model. The trained ANN model is then implemented on the PIC. The details of the ANN model developed in this work can referred to previous publication [7] [8].

Results and Discussions

In this work, the changes in the concentration of H_2S and temperature were analyzed statistically using two-sample t-test. 100 data was randomly selected from two groups of recorded data. The group of data that had been collected from the preliminary investigation run with the sample containing SRB is referred as treatment group, whereas the data collected from the samples without bacteria was labeled as control group. The t-test evaluates the difference between the means of two populations. In this work, the t-test was conducted with 95% (alpha level = 0.05) of confidence level.

The results obtained from the analyses are summarized in Table 1. The computed p-value from the t-test is 0.00, which is less than pre-specified alpha level. The investigation results statistically evidences that under the presence of SRB, the concentration level of H_2S and temperature are significantly different. Thus, the data recorded can be used in the ANN training to detect and identify the presence of SRB.

Sample	Ν	Mean		Std. dev.	Std. dev.	
		Volt.	Temp.	Volt.	Temp.	
With SRB	50	1.7395	42.58	0.08	0.48	
Without SRB	50	0.9136	39.18	0.06	0.52	
P-value		0.00	0.00	-	-	

Table 1. Results of Two-sample T-test Analyses

The performance of the overall system is typically evaluated by computing the percentage of accuracy in detecting the presence of SRB in a sample. In the present work, 40 samples were randomly selected and presented to the system. The results show that the system achieve promising performance in classifying the samples into two groups, either with SRB or without SRB, with 97% classification rate.

Table 2	Results	of ANN	for the	real-time	detection	of SRR
Iuoic 2.	mesuus (j_{AIVIV}	joi inc	reui-iime	ueiecnon	UJ SKD

Samples	Predicted as samples	C	
	With SRB	Without SRB	Success rate
With SRB	18	1	94.73%
Without SRB	0	21	100%
Overall Success Rate			97.73%

Conclusions

In this article, a PIC-based electronic prototype system that can detect the presence of SRB is presented. The achieved results support the proposed framework as a promising technique to detect the presence of SRB faster than traditional culture-based approach. However, the design merely represents the first step toward creating a robust system that able to perform on-site measurement. The project can be further expanded by connect several sensor nodes which located at different places to the Internet, and using the network infrastructure to analyze the data and generate useful information to help companies to monitor and manage the risks of corrosion caused by SRB.



Earn Tzeh Tan

AASCIT member (http://www.aascit.org/membership/etspace). PhD student in School of Electrical and Electronic Engineering, Universiti Sains Malaysia, Penang, Malaysia. Research interests include embedded system applications and machine learning algorithms. tanearntzeh@yahoo.com

References

- [1] World Corrosion Organization. (2014) Corrosion Costs and the Future. http://corrosion.org.
- [2] Ghazy, E. A., Mahmoud, M. G., Asker, M. S., Mahmoud, M. N., Abo Elsoud, M.M., and Abdel Sami, M. E. (2011) Cultivation and Detection of Sulfate Reducing Bacteria (SRB) in Sea Water. Journal of American Science. 7, pp. 604-608.
- [3] Postgate, J. R. (1984) The Sulphate reducing bacteria. Cambridge: Cambridge University Press.
- [4] Biosan Laboratories, INC. (2014) Sani-Check SRB: Test Kit for Counting Sulfate Reducing Bacteria. https://www.biosan.com/sulfate-bacteria-test-kit.
- [5] Rastogi, G., and Sani, R. K. (2011) Molecular Techniques to Assess Microbial Community Structure, Function and Dynamics in the Environment. Microbes and Microbial Technology: Agricultural and Environmental Applications. Springer.
- [6] Eitan, B. D., Brenner, A., and Kushmaro, A. (2007) Quantification of Sulfate-reducing Bacteria in Industrial Wastewater, by Real-time Polymerase Chain Reaction (PCR) Using dsrA and apsA Genes. Microbial Ecology. 54, pp. 439-451.
- [7] Tan, E. T., Abdul Halim, Z., Darah, I., Abdul Rahim, R., Mohamad Saleh, J., and Chandran, U. D. (2012) Artificial Neural Network-based Electronic Nose for the Detection of Sulfate-reducing Bacteria. Sensors and Transducers. 17, pp. 50-59.
- [8] Tan, E. T., and Abdul Halim, Z. (2012) Development of an Artificial Neural Network System for Sulphate-reducing Bacteria Detection by using Model-based Design Technique. 2012 IEEE Asia Pacific Conference on Circuits and Systems (APCCAS 2012). Kaohsiung, Taiwan, pp. 352-355.