

E-Nose System to Detect E-Coli in Drinking Water of Udupi District

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Abstract— E-nose (Electronic nose), which is an array of chemical sensors, is widely used in food industry and pollution control. Nowadays e-nose technology has become powerful tool to evaluate the aroma compounds during the quality control process. This work is intended to detect biological water contaminants like coliform group of bacteria in drinking water. However, a number of limitations are associated with traditional methods of microbial detection. The water samples were collected from the River Swarna in Udupi district and analyzed for the presence of E-coli. An E-nose system is developed using embedded PIC micro controller to detect and quantify the microbial contaminants. The results of this analysis were compared with standard Multiple Test Tube Fermentation (MTTF) method, which indicates the Most Probable Number (MPN) for the bacterial density in drinking water.

Keywords—E-nose, E-coli, PIC Micro Controller, Multiple Test Tube Fermentation (MTTF) method, Most Probable Number (MPN)

I. INTRODUCTION

E-nose are being developed as systems for the automated detection and classification of odour, vapors and gases. The detection of gases using e-nose has a wide range of application in air quality monitoring [1], food spoilage detection [2] and medical diagnostic [3]. E-nose contains array of sensors resulting in multiple measurements which have been analyzed using pattern recognition techniques. An e-nose could be a universal vapor sensor, in practice however all existing e-nose detects only a family of related chemicals due to the difficulty of producing a variety of sensing materials required for universal sensing [4]. E-nose using SnO₂ gas sensors are used for detection of methanol contamination in whiskeys [5]. In this work, we detect E-coli in drinking water using CO₂ sensor. The water is collected from Swarna river of Udupi district. Swarna river, which is born in Agumbe Ghats and flows through Udupi district, reaches Arabian sea. Swarna river water is the main source of drinking water for people residing in and around Manipal and Udupi.

Drinking water must possess high degree of purity and free from micro-organism. The potential and quality of drinking water is an economic resource and essential component for survival of living beings on earth. Living conditions are being deteriorated in major rural centers due to pollution caused by population explosion, urbanization and microbial contaminations [7]. The detection of microorganisms in drinking water that are harmful to health, gives a historical overview of the risk of disease from pathogens. E.coli is the most abundant coliform organism present in the human [8] and animal intestine, and can be present in faeces causing a main concern to the health. Since E.coli and other coliform organisms are present in large numbers in faeces and sewage, they can be detected in numbers as small as 1 in 100 ml of water. They are considered to be the most sensitive indicator bacteria for demonstrating excremental contamination. For this reason, quantification of E.coli in drinking water is important to assess the degree of pollution and thereafter to protect human health [6].

Traditionally indicator microorganisms have been used to suggest the presence of pathogens. The specific methods for the detection and enumeration of enteric pathogenic bacteria and viruses are complex and time consuming [11]. The test for detection and enumeration of indicator organisms, rather than pathogens are used.

A. Assessment of Water Quality by Detecting Microbial Water Contaminants

The commonly used methods to detect the presence of coliform bacteria are Membrane Filter technique and Multiple Test Tube Fermentation (MTTF) technique. Membrane filter technique involves direct plating for detection and estimation of coliform densities [7]. The toxic substances in the water may be absorbed by filter and interfaces with bacterial growth. The plate count provides an approximate enumeration of total number of bacteria multiplying at room temperature that provide approximate information about water quality. Thus, the membrane filter technique fails to detect and quantify bacterial count in drinking water. MTTF technique involves mixing different dilutions of a sample of water with lactose broth and incubating them in test tubes for 48 hours at room temperature. The presence of CO₂ gas tubes will indicate the presence of coliform bacteria. Most Probable Number (MPN) represents the bacterial density [11]. The presence or absence test using MTTF method is used to monitor high quality samples, where the presence of the target organism is improbable. The sensitivity of this technique depends on the sample volume analyzed and the precision on the number of samples analyzed in parallel with each dilution step. In all of the enumeration techniques, the cultivation conditions are selected to

promote the multiplication of the target organisms, while simultaneously inhibiting the growth of other organisms. The balance between sensitivity and selectivity is the reason for different methods for drinking water and highly contaminated waters. Since in our work we are concentrating on drinking water, MTTF method is preferred. The MPN index represents the bacterial density.

II. METHODOLOGY

Multiple Test Tube Fermentation (MTTF) method is performed with 100ml of water sample using three test tubes, containing serial dilution, that are incubated in to a broth media. The combinations of positive results are interpreted from statistical tables, to estimate the number of organisms present. After incubating water sample with media for 24 to 48 hours the MPN Index is calculated. If not more than 1 coliform is present per 100ml of water, then water is said to be safe for drinking. The experimental set up for MTTF test with e-nose system is shown in Fig.1.



Fig. 1 Experimental setup

The MTTF method, also referred as MPN method, is based on an indirect assessment of microbial density in the water sample by reference to statistical tables, to determine the MPN of organism present in the original sample. The MPN per 100ml of sample using three test-tubes of each dilution is recorded from the MPN (Table I). [APHA-AWWA-WPCF, 'Standard methods for the examination of water and wastewater']

The water samples collected for bacterial analysis are: Sample 1: Water collected directly from Swarna river during rainy season; Sample 2: Water collected directly from Swarna river during summer season; Sample 3: Water collected after preliminary filtration (Swarna river water); Sample 4: Filtered and chlorinated drinking water supplied from Udupi Municipal Corporation (Swarna river water).

The coliform group of bacteria is detected based on lactose fermentation. The release of CO₂ gas at room temperature proportional to the bacterial count is the indication of bacterial contamination. To improve culture-based techniques for monitoring specific microbial populations in water samples, the current study has adapted the sensor technology [7] to automate the process of water analysis.

TABLE I MPN TABLE PER 100 ML OF SAMPLE

Number of positive tubes in dilutions			MPN per 100 ml	Number of positive tubes in dilutions			MPN per 100 ml
10 ml	1 ml	0.1 ml		10 ml	1 ml	0.1 ml	
0	0	0	0	2	0	0	9.1
0	1	0	3	2	0	1	14
0	0	2	6	2	0	2	20
0	1	0	3	2	0	3	26
0	1	1	6.1	2	1	0	15
0	1	2	9.2	2	1	1	20
0	1	3	12	2	1	2	27
0	2	0	6.2	2	1	3	34
0	2	1	9.3	2	2	0	21
0	2	2	12	2	2	1	28
0	2	3	16	2	2	2	35
0	3	0	9.4	2	2	3	42
0	3	1	13	2	3	0	29
0	3	2	16	2	3	1	36
0	3	3	19	2	3	2	44
1	0	0	3.6	3	0	0	23
1	0	1	7.2	3	0	1	39
1	0	3	11	3	0	2	64
1	1	0	7.3	3	0	3	95

1	1	1	11	3	1	0	43
1	1	2	15	3	1	1	75
1	1	3	19	3	1	2	120
1	2	0	11	3	1	3	160
1	2	1	15	3	2	0	93
1	2	2	20	3	2	1	150
1	2	3	24	3	2	2	210
1	3	9	16	3	2	3	290
1	3	1	20	3	3	0	240
1	3	2	24	3	3	1	460
1	3	3	29	3	3	2	1100

The coliform group of bacteria is detected and quantified using PIC microcontroller based data acquisition system (DAQ). Fig. 2 shows the simplified schematic diagram of the e-nose system. The project flow is shown in Fig. 3.

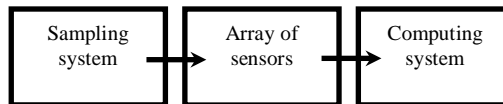


Fig.2. Simplified schematic diagram of e-nose system

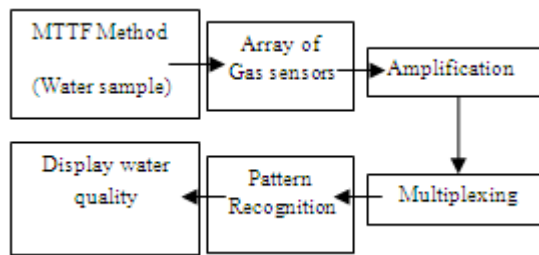


Fig.3 Block diagram of e-nose system

The e-nose system involves hardware and software implementation. The water sample is tested using MTF method and the CO₂ gas released depending on the bacterial contamination, is sensed using array of nine carbon dioxide sensors. Signal conditioning circuit using OPAMP will improve the quality of signals. The sensor output voltage is measured using an operational amplifier with high input impedance and low bias current (less than 1 pA) shown in Fig. 4.

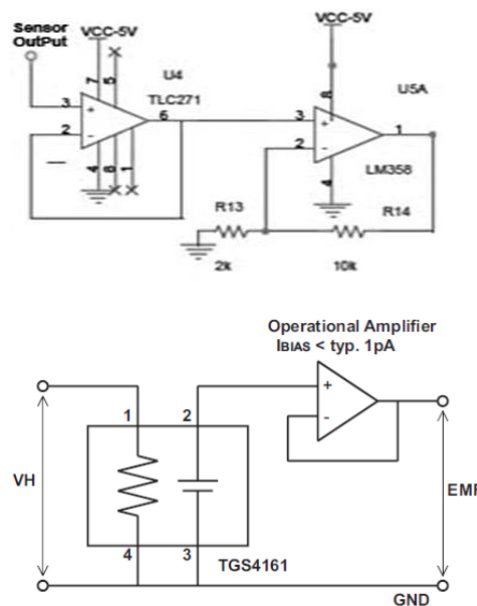


Fig.4 Sensor interface and signal conditioning

The PIC Microcontroller is programmed to detect and quantify the microbial water contaminants as shown in below flow diagram Fig. 5. The schematic diagram of e-nose system is built using PSPICE as shown in Fig. 6.

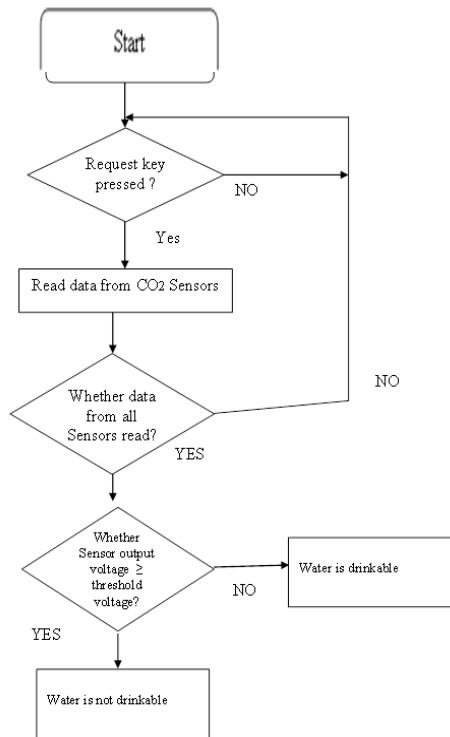


Fig.5 Software flow diagram

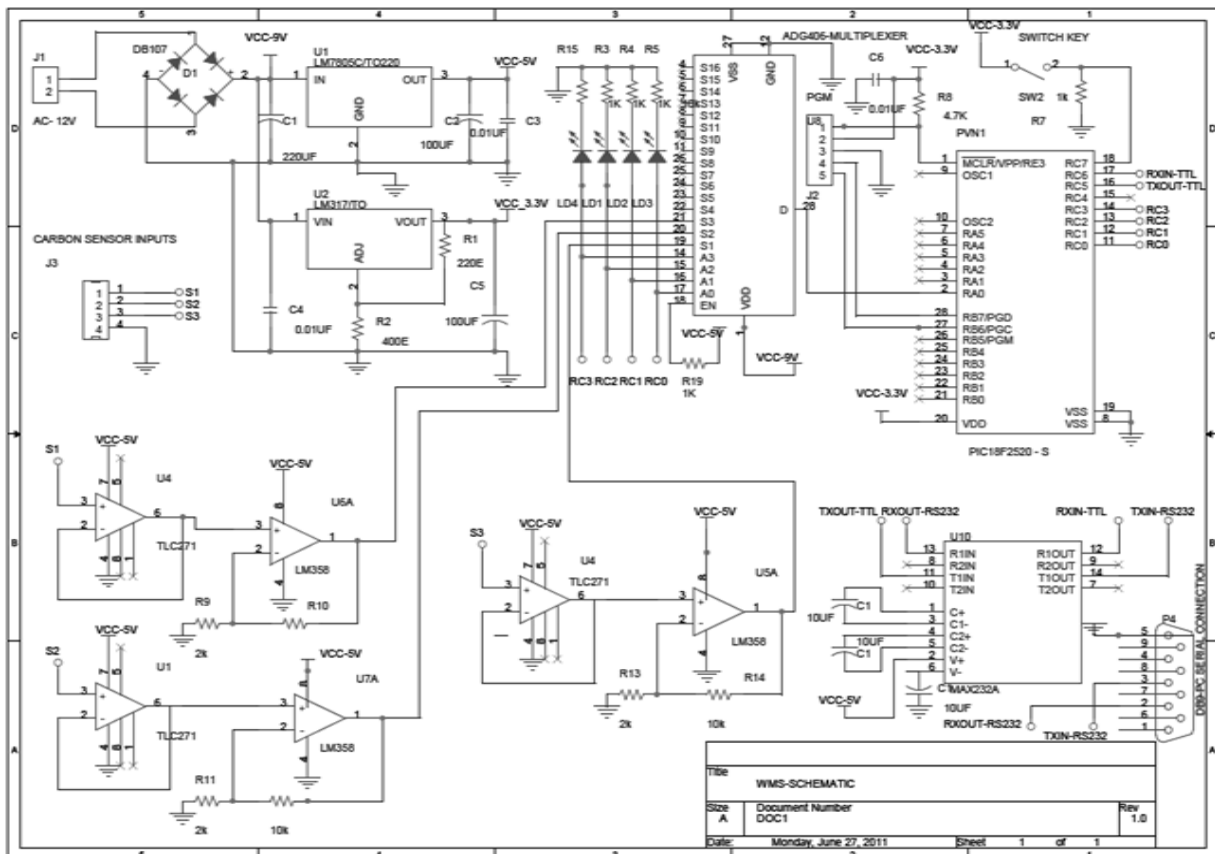


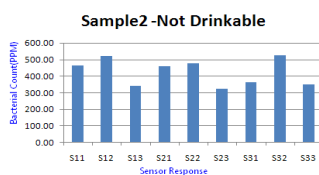
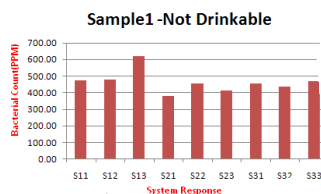
Fig.6. Circuit diagram of e-nose system

III. EXPERIMENTAL RESULTS

The MPN index of Bacterial count is recorded from statistical table and it is compared with sensor response of e-nose system as shown in Table II. The sensor response 220 mV for 350 ppm of CO₂ gas is taken as threshold voltage. The tables prove that e-nose system accurately measures microbial water contaminants and indicates the degree of contamination. The bacterial concentration for all 4 samples are plotted as shown in Fig. 7, which indicates that bacterial count varies randomly and This system is able to monitor the degree of contamination and thus the water quality.

TABLE II : TABLE OF BACTERIAL COUNT PER 100 ML OF SAMPLE USING THREE TEST-TUBES OF EACH DILUTION

Water sample	MPN No (Index)			MPN Index	Sensor response			Percentage Water Quality	Comment
	No of positive tubes out of 3				10ml	1ml	0.1ml		
	10 ml	1 ml	0.1 ml		S ₁₁ S ₁₂ S ₁₃	S ₂₁ S ₂₂ S ₂₃	S ₃₁ S ₃₂ S ₃₃		
Sample1 (collected from river during rainy season)	3	3	2	1100/100ml	299m V 300m V 390m V	240m V 285m V 260m V	285m V 275m V 295m V	0%	Not Drinkable
Sample 2 (collected from river during summer)	2	2	2	35/100ml	293m V 328m V 215m V	289m V 300m V 205m V	229m V 329m V 219m V	25%	Not Drinkable
Sample 3(after preliminary filtration)	1	2	2	20/100ml	282m V 210m V 200m V	280m V 260m V 199m V	500m V 290m V 203m V	50%	Treatment required
Sample 4(filtered and chlorinated by municipal corporation for drinking purpose)	0	0	0	0/100ml	120m V 118m V 110m V	115m V 116m V 115m V	105m V 108m V 110m V	100%	Drinkable



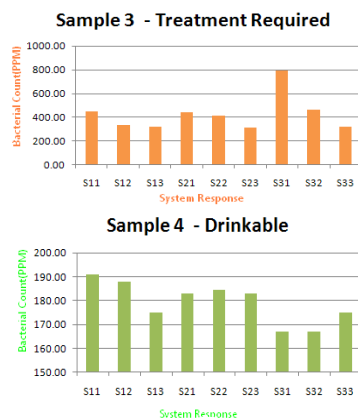


Fig.7. E-nose system responses for different water samples.

IV. CONCLUSION

E-nose system found to be effective in detecting and accurately quantifying the microbial contaminants, specifically coliform group of bacteria (E. coli). This work compares the traditional bacterial count in MPN index with sensor response. The system monitors the quality of drinking water and display the percentage level of microbial contaminants. Further improvement in the system can be done by integrating advanced chemical sensors for detecting chemical contaminants and hardness of the drinking water.

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Biography



K. M. Nayak obtained his BE in Electronics and Communication Engineering from Mangalore University, Karnataka India and MTech in Industrial Biotechnology from Dr.MGR University Chennai. He is the Associate member of Institute of Engineers India and Life member of Indian society for Technical Education. Currently he is working as Assistant Professor (Sel. Grade) in Department of Electronics and Communication Engineering, MIT, Manipal of Manipal University and part time research scholar at Dr. MGR University, Maduravoyal, Chennai. His specialization includes Bio MEMS and Microsensor and Bio Electronics. His current research includes E-nose technology for Environmental Monitoring and Artificial Neural Networks.