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A constant phase element sensor for monitoring microbial growth

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Abstract

The construction and performance of a novel constant phase element (CPE) sensor for monitoring microbial growth has been reported in this paper. The signal conditioning circuit measures the changes in the phase angle of the impedance characteristics of the probe. The major advantages of the measurement system developed are the simplicity of the construction of the probe and ease in measurement. It is proposed that the sensor can be effectively used for monitoring bacterial and yeast growth.

Keywords: Constant phase element (CPE); Microbial growth; Phase sensitive detector; Yeast culture; Spoilage of milk

1. Introduction

Impedance spectroscopy has now emerged as a powerful technique for in situ monitoring of complex electrochemical processes [1]. Recent studies have reported the existence of constant phase behaviour in few electrode–electrolyte combinations [1–4].

A constant phase element (CPE) essentially has the impedance whose phase angle remains constant over a wide range of frequencies. An ideal resistor and an ideal capacitor are two examples of constant phase element whose phase angle remains constant at 0° and −90°, respectively [4]. In recent past, development of CPE with a phase angle in between these two extreme values has been reported [5]. The construction and performance of the CPE based on poly-methyl-methacrylate (PMMA) has been reported in [6]. The major feature of this probe is simplicity in construction. Its phase characteristics also remain fairly *constant* over a wide range of frequencies. It has also been observed that the phase angle can be varied over a considerable range, depending on the ionic concentration of the polarizable liquid where the probe is dipped in. This observation has led to the concept of development of a new kind of 'CPE sensor' that will provide a phase angle, dependent on the ionic concentration of the liquid medium. The performance of the sensor for monitoring microbial growth in yeast and raw milk has been proposed in this paper.

The impedance technique to detect microbial growth is more than 100 years old. The basic principle is to sense the change of electrical characteristics of the medium where microbial growth takes place [7]. The signal is expressed as a curve similar to the microbial growth curve, as obtained by other methods reported in literature, is a rapid and sensitive means of detecting active microorganisms. As this technique has a potential basis of a rapid automated system in the field of microbiology, several researchers have proposed different kinds of impedance measuring technique under the umbrella of impedance microbiology [8–13].

Food technology has adopted impedance microbiology to detect the microbial activity in food and its wide application can be noticed in dairy industry [9,11,14–16]. In 1978, Cady [14] proposed the impedance as an alternative method to replace the plate count for rapid screening of milk microbial content. Felice et al. [9] proposed a capacitance growth curves to quantify the bacterial content in milk. Keat et al. [15] proposed a resonant-circuit sensor based on an LC resonator for monitoring of bacterial growth. A dielectric technique for monitoring yeast cell division has been reported [8].

In impedance technique the measurement is essentially carried out in three different ways: measurement of (i) equivalent conductance of the probe, (ii) equivalent capacitance, or (iii) the total impedance across the two terminals [7,9]. In all the cases, the measured values are dependent on the frequency. Besides

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the measuring circuit for the first two methods are complex. All these difficulties can be avoided if instead a CPE probe is used. Characteristics of a CPE sensor differ from a conventional 'Impedance Sensor' in a way that the phase angle introduced by the Sensor is measured (as in the present case); as a result the measuring circuit becomes simpler.

In yeast culture, monitoring the pH level of the substrates is an essential parameter to detect the optimal growth of the cell [17–21]. In an uncontrolled batch process, the carbon dioxide and H^+ ions released from the cell growth reduces the pH level of the medium. This drop in pH, unless controlled, would affect further yeast growth.

In the present case, it has been observed that the proposed sensor system in the medium can effectively monitor the variation of H^+ ion concentration. A personal computer (PC) based on-line measurement scheme has been used and it has been observed that the output of the system closely resembles the bacterial and yeast growth curve reported in literature.

The major advantage of the scheme is that only a single probe is involved in the sensing which is rigid and can be easily dipped inside the culture medium. The measuring circuit is also very simple. As the phase angle is constant for almost one decade of frequency, frequency setting is not a big problem in the measurement which remained a major concern in the impedance microbiology [9,13,15].

2. CPE construction and behaviour

In a previous work the authors reported the construction and fabrication process of a CPE as shown in Fig. 1 [6,22]. The probe shown in Fig. 1 is made of about 0.5 mm wide strip cutout from about a 1.62 mm thick copper plate that is generally used as double sided printed circuit boards (PCB). The height of the probe is 8 cm. The copper claddings on the two external faces serve as the electrodes of the probe. The insulation coating on the copper electrodes is provided by inserting the probe into 2.5% solution of PMMA, by dissolving PMMA in chloroform solvent; and this results about a 5 μm PMMA coating on the copper electrodes at both sides of the probe, as shown in Fig. 1.

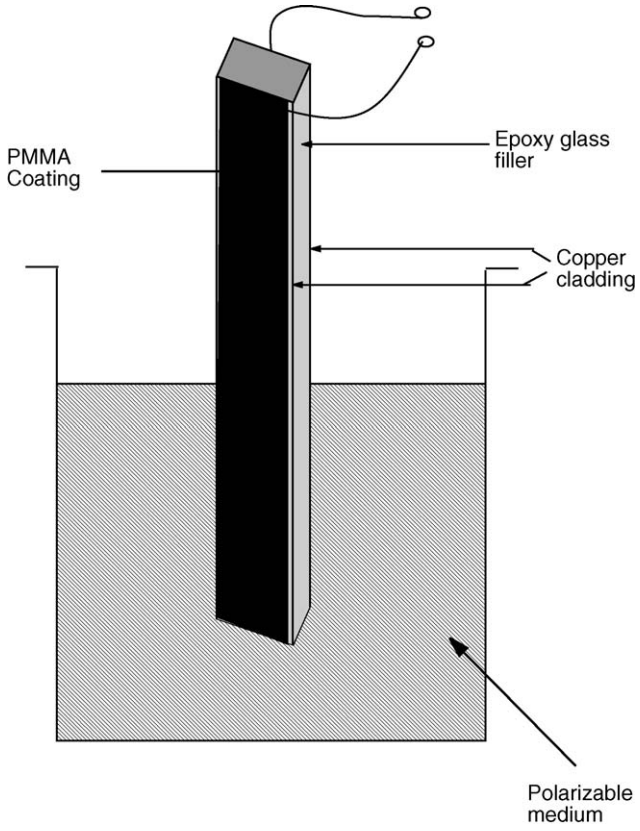

Fig. 1. Construction of the CPE.

The above arrangement gives constant phase angle for almost one decade of frequency. The impedance Z of the CPE can be represented as [3–5]

$$Z_{\mathrm{CPE}} = Qs^{-\alpha}, \tag{1}$$

where s is the Laplace operator. Putting $s = j\omega$, where ω is the frequency in radian/sec, we obtain $|Z_{\mathrm{CPE}}| = Q\omega^{-\alpha}$ and $\angle Z_{\mathrm{CPE}} = \theta = -\pi\alpha/2$ radians. The angle θ is independent of frequency.

The coefficient Q and the fractional exponent α are the parameters of the CPE. Generally $-1 < \alpha < 1$. The dependence of the constant phase angle θ on various parameters have been investigated experimentally and from the experimental observation it can be said that

$$\theta_{\mathrm{CPE}} = f(A, t, \sigma), \tag{2}$$

where A is the area of contact of the electrodes with the polarizing medium, t the thickness of the insulation on the electrodes and σ is the ionic concentration of the polarizing medium.

The dependence of the constant phase angle on different parameters in Eq. (2) has been tested. The measurement is done with a Agilent precision Impedance Analyzer (model No. 4294A) in Z, θ mode, excited with a sinusoidal signal of 1 V peak to peak in the frequency range of 1 kHz to 1 MHz [6,22]. In all the cases, the probe has been dipped to a length of 1 cm inside the medium. Fig. 2 shows the behaviour of probes constructed by varying coating thickness, length and width; with tap-water as the medium. Different phase angles varying between $-20°$ to $-83°$ can be obtained and the phase angles remained constant for almost one decade of frequency. The data 5 plot shows this characteristics for the probe used in the present microbial detection. From the plots it can be seen that the frequency range of constant phase angle for the probes 3–5 is almost from 20 to 200 kHz, while for probes 1 and 2 the constant phase behaviour starts at the frequency about 100 kHz and extends upto 1 MHz.

The dependence of the phase angle on the ionic concentration has been investigated through two different experiments. In the first one, the probe is dipped 1 cm inside KCl solutions with different concentrations. The change in phase angle with varying frequency is shown in Fig. 3. In the second case, standard pH buffer tablets are used to prepare solutions of different pH values. The behaviour of the probe when dipped in the solutions are plotted in Fig. 4. In both the cases, the probe dimensions originally reported in the beginning of this section has been used.

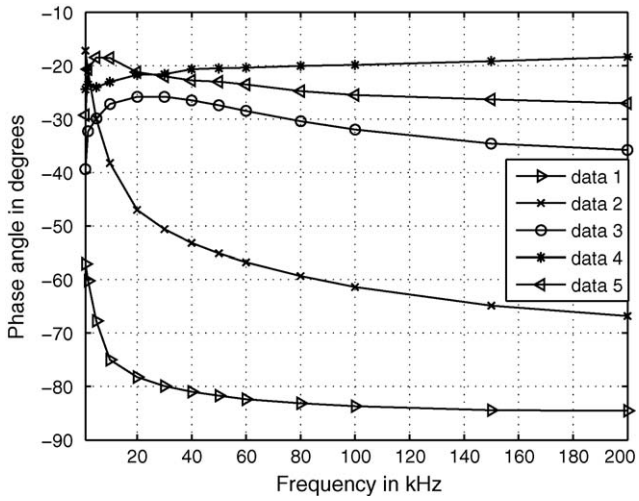

Fig. 2. Behaviour of constant phase angle characteristics of probes constructed with varying coating thickness, length and width when immersed 1 cm in tap-water; data 1: coating thickness = 47 μm, probe length = 12 cm, probe width = 6 mm; data 2: coating thickness = 28 μm, probe length = 2 cm, probe width = 1 cm; data 3: coating thickness = 12 μm, probe length = 2 cm, probe width = 6 mm; data 4: coating thickness = 5 μm, probe length = 2 cm, probe width = 1 cm; data 5: coating thickness = 5 μm, probe length = 8 cm, probe width = 0.5 mm (used in microbial detection).

From Figs. 3 and 4 it is apparent that the phase angle of the impedance of the probe changes with the ionic concentration, and the phase angle also remains fairly constant over a wide range of frequencies. It is to be noted that the constant phase angle behaviour cannot be obtained with a finite number of series-parallel R–C combinations [23]; and as a result, the CPE has a separate identity compared to other passive devices. In the following sections, we shall look into the possibility of using the CPE as a sensor for monitoring microbial activities.

It is a well known fact that metabolic activity of microorganisms transforms the low conductive substrate/nutrients (carbohydrate, peptide proteins) into high conductive components [7,24] which cause the ionic change in the culture medium. The

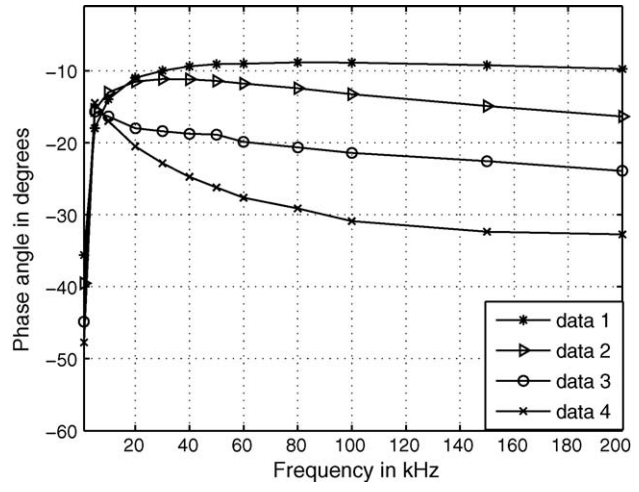

Fig. 3. Variation of phase angle with conductivity of the medium; data 1: N/4 KCl solution (conductivity = 39 mmho/cm), data 2: N/32 KCl solution (conductivity = 4.9 mmho/cm), data 3: N/128 KCl solution (conductivity = 0.9 mmho/cm), and data 4: N/1024 KCl solution (conductivity = 118 μmho/cm).

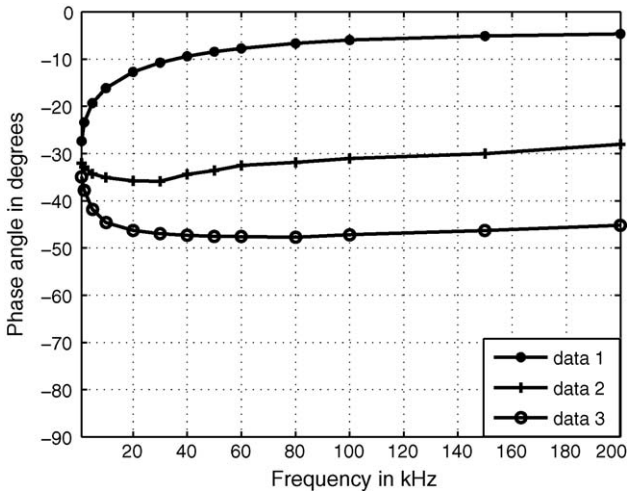

Fig. 4. The constant phase angle behaviour in three different pH solutions; data 1: pH 4, data 2: pH 7, and data 3: pH 9.2.

probe being covered with a polymer film is harmless to microorganisms. And several reports can be found which state that the pH of the extra cellular medium decreases with time as yeast grows. This can be the by-product of a few different mechanisms [17,18]. So, a CPE sensor can be constructed by dipping the probe inside the culture medium in which the phase angle will change with the progress in microbial reaction in the culture medium. A typical observation for the phase angle change from milk to curd has been obtained as shown in Fig. 5.

3. Schematic arrangement to detect the phase angle change

The phase detector circuit [25] is shown in Fig. 6. Sinusoidal excitation of 1 V peak to peak at 50 kHz has been applied. One of the two amplifiers, at the first stage of the circuit, gets input directly from the signal generator and acts as a reference signal. The signal for the other amplifier comes through the CPE which is dipped inside a polarizable medium. These signals are

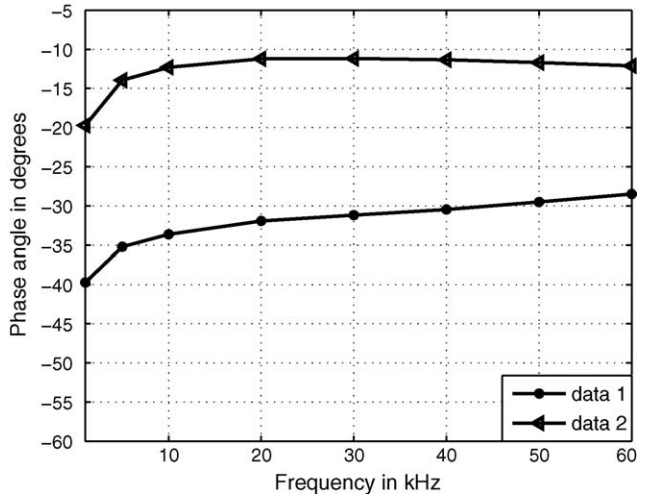

Fig. 5. Constant phase angle behaviour of the probe in milk and curd; data 1: dipped 1 cm inside milk and data 2: dipped 1 cm inside thick curd.

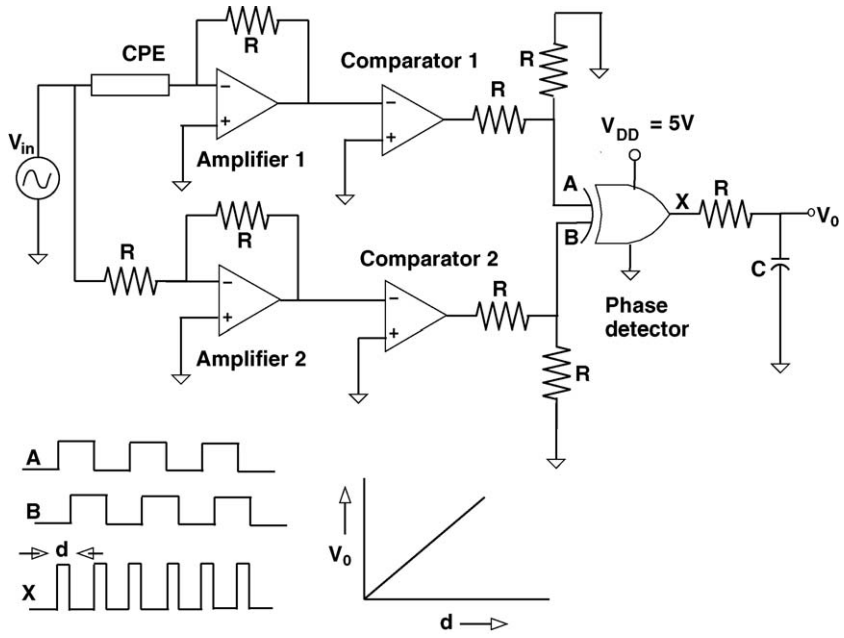

Fig. 6. Circuit to detect the phase angle change.

Table 1
The voltage output of the circuit at three different pH values

pH	Output voltage (V)
4	0.377
7	2.00
9.2	3.49

amplified and fed to the comparators which convert the sinusoidal signal to a square wave. The digital phase detector is constructed with XOR gate as indicated in Fig. 6. From the truth table of the XOR gate it can be seen that when d, the phase difference between the two inputs of the XOR gate, is zero, the output voltage $v_0 = 0$; and when the phase difference is 90°, it is maximum (half of the V_{DD} applied to the XOR gate chip). In between it gives an output voltage proportional to the phase difference between the two inputs which is a measure of the ionic concentration of the polarizing medium in which the CPE is dipped in. The performance of the measuring system with different pH solutions are given in Table 1.

4. Experiment

Experiments have been carried out to monitor growth of yeast cells and spoilage of raw milk using the measuring scheme. The output of the phase detector circuit is interfaced with a PC and the data are stored over several hours in an interval of 1 min. The preparation of the medium and the variation of the output are reported below. All the experiments are carried out at room temperature (26–30 °C).

4.1. Yeast culture

Dry yeast cells are activated in a culture medium. To prepare the medium glucose, malt extract, peptone and yeast extract are mixed in deionized water at a ratio of 10:5:3:3 gm/l, respectively. Then a pinch of dry yeast cell and 0.85 gm of saline is put in 100 ml of the prepared culture medium. The medium is kept inside an autoclave at 37 °C at 120 rpm for 3–4 h so that the dry yeast cell gets activated. The activated yeast cell is added to a culture medium as described above at a ratio of 1:10 [26]. The probe is dipped inside the culture medium and connected to the phase detector circuit to measure the ionic change due to the microbial and biochemical activity in the medium. The culture medium is stirred in every 1 h with a sterilized glass rod to provide sufficient oxygen into the culture. The output voltage curve corresponding to the ionic change in the medium is shown in Fig. 7.

4.2. Spoilage of raw cow milk

In the present experiment 250 ml of raw cow milk is taken in a sterilized glass jar. The probe is dipped 1 cm inside the milk and left undisturbed approximately at 27 °C. The CPE is connected to the phase detector circuit shown in Fig. 6. The output voltage curve is shown in Fig. 8.

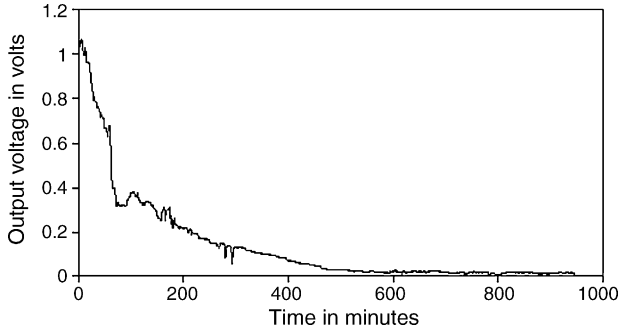

Fig. 7. The output voltage curve showing the ionic change in the yeast culture medium.

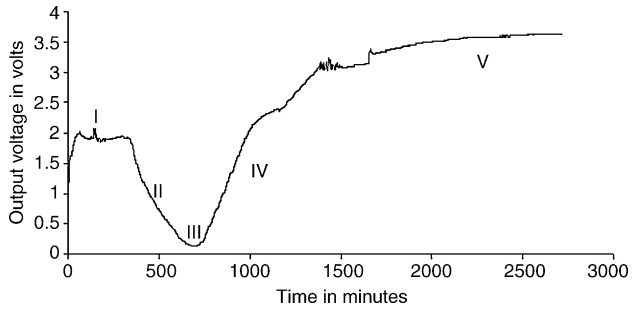

Fig. 8. The output voltage curve showing the ionic change of the medium due to microbial reaction leading to spoilage of raw cow milk.

5. Results and discussions

It can be seen from Fig. 7 that in case of yeast culture the output voltage is decreasing monotonically with time and finally settling to a steady state one. This indicates the decrease in phase angle of the CPE sensor in the phase detector circuit. Comparing with the result shown in Fig. 4, one can conclude the monotonic drop in the pH value of the medium (as lesser pH value gives lesser phase angle difference, hence, lesser *d*). This is attributed to the release of carbon dioxide and H^+ ions, during the cell growth. The logistic growth model (LGM) is widely used for describing the growth of the yeast cells [21]. An inflection point is normally observed in the cell concentration versus time graph [17,27], depicting the 'S'-type nature of the curve. But this inflection time is not always visible when the cell concentration is plotted in log scale. We also do not observe any inflection point in Fig. 7, that can be justified by noting the implicit log scale present in the pH scale. Thus, Fig. 7 can truly represent the growth of yeast cells in the medium.

In the second case, the spoilage of raw cow milk over a period of time has been observed using the proposed scheme in Fig. 8. There is a definite lag phase present here denoted by 'I' in Fig. 8. In second phase ('II') the pH of the solution decreases steadily and then in third phase ('III') it remains constant. In fourth phase ('IV') pH starts to increase which indicates the decrease of conductivity of the reactive medium and finally in fifth phase ('V'), it becomes constant. The corresponding output voltages are indicated by *Y*-axis and comparing these values with Table 1 the pH values of the solution can be obtained. The nature of the curve closely resembles the pH variation curve in microbial population, as shown in Fig. 9.

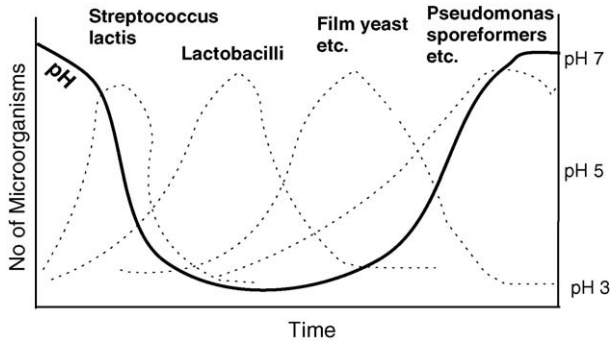

Fig. 9. Succession of species in raw milk at room temperature; here redrawn from [17].

6. Conclusion

Though the existence of constant phase behaviour at the metal-insulator-polarizable medium is known to the researchers in electrochemistry, but the concept of a CPE sensor is not common. The present paper proposes for the first time the construction of a CPE sensor, the signal conditioning circuit and its application to monitor the microbial growth. The major advantage is that the sensor is inert, cheap and robust, and can be interfaced with the automated system easily. Thus the proposed method may find ready applications for on-line monitoring of growth of several processes.

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References

[1] J.R. Macdonald, Impedance Spectroscopy Emphasizing Solid Materials and Systems, John Wiley and Sons, 1987.
[2] R. Greef, R. Peat, L.M. Peter, D. Pletcher, J. Robinson, Instrumental Methods in Electrochemistry, Ellis Horwood Series in Physical Chemistry, New York, 1990.
[3] M. Itagaki, A. Taya, K. Watanabe, K. Noda, Deviation of capacitive and inductive loops in the electrochemical impedance of a dissolving iron electrode, Jpn. Soc. Anal. Chem. 18 (2002) 641–644.
[4] P. Zoltowski, On the electrical capacitance of interfaces exhibiting constant phase element behavior, J. Electroanal. Chem. 443 (1998) 149–154.
[5] J. Bisquert, G. Garcia-Belmonte, F. Fabregat-Santiago, N.S. Ferriols, Doubling exponent models for the analysis of porous film electrodes by impedance: relaxation of TiO_2 nanoporous in aqueous solution, J. Phys. Chem. 104 (2000) 2287–2298.
[6] K. Biswas, S. Sen, P.K. Dutta, Realization of a constant phase element and its performance study in a differentiator circuit, communicated to IEEE Transactions of Circuits and Systems-II, 2005.
[7] A. Ur, D.F. Brown, Impedance monitoring of bacterial activity, J. Med. Microbiol. 8 (1975) 19–27.
[8] K. Asami, E. Gheorghiu, T. Yonezawa, Real-time monitoring of yeast cell division by dielectric spectroscopy, Biophys. J. 76 (1999) 3345–3348.
[9] C.J. Felice, R.E. Madrid, J.M. Olivera, V.I. Rotger, Impedance microbiology: quantification of bacterial content in milk by means of capacitance growth curves, J. Microbiol. Meth. 35 (1999) 37–42.
[10] J.D. Owens, D.S. Thomas, P.S. Thomas, J.W. Timmerman, Indirect conductimetry: a novel approach to the conductimetric enumeration of microbial population, Lett. Appl. Microbiol. 9 (1989) 245–249.
[11] A. Stolle, B. Schalch, H. Eisgruber, Impedance microbiology: application in food hygiene, J. Food Prot. 62 (1999) 1488–1496.
[12] L. Yang, C. Ruan, Y. Li, Detection of viable salmonella typhimurium by impedance measurement of electrode capacitance and medium resistance, Biosens. Bioelectron. 19 (2003) 495–502.

[13] R. Gomej-Sjorberg, R. Bashir, A. Bhunia, Microscale electronic detection of bacterial metabolism, Sens. Actuators B: Chem. 86 (2002) 198–208.
[14] P. Cady, D. Hardy, S. Martins, S. Dufour, S.J. Kraeger, Automated impedance measurement for rapid screening of milk microbial count, J. Food Prot. 41 (1978) 277–283.
[15] K.G. Ong, J.S. Bitler, C.A. Grimes, L.G. Puckett, L.G. Bachas, Remote query resonant-circuit sensors for monitoring of bacteria growth: application to food quality control, Sensors 2 (2002) 219–232.
[16] P. Neaves, M.J. Waddel, G.A. Prientice, A medium for the detection of lancefield group D Cocci in skimmed milk powder by measurement of conductivity changes, J. Appl. Bacteriol. 65 (1988) 437–448.
[17] J.E. Bailey, D.F. Ollis, Biochemical Engineering Fundamentals, McGraw-Hill Book Company, Singapore, 1986.
[18] D.A. Knorre, E.A. Smirnova, F.F. Severin, Natural conditions including programmed cell death in the yeast saccharomyces cerevisiae, Biochemistry (Moscow) 70 (2005) 264–266.
[19] A. Rose, Physiology and Biochemistry of Yeasts, 2nd ed., Academic Press, London, England, 1971.
[20] M.L. Shuler, F. Kargi, Bioprocess Engineering Basic Concepts, 2nd ed., Prentice Hall, Englewood Cliffs, NJ, 2001.
[21] A.S. Vadasz, P. Vadasz, M.E. Abashar, A.S. Gupthar, Recovery of an oscillatory mode of batch yeast growth in water for a pure culture, Int. J. Food Microbiol. 71 (2001) 219–234.
[22] K. Biswas, S. Sen, P.K. Dutta, Modelling of a capacitive probe in a polarizable medium, Sens. Actuators A: Phys. 120 (2005) 115–122.
[23] S.C.D. Roy, On the realization of a constant-argument immitance or fractional operator, IEEE Trans. Circuit Theory (1967) 264–274.
[24] K. Todar, Growth of bacterial population, Todar's Online Textbook of Bacteriology, http://textbookofbacteriology.net.
[25] D. Wobschall, Circuit Design for Electronic Instrumentation, McGraw-Hill, USA, 1979.
[26] G. Saha, Time delay control for bioprocesses and its application to a newly developed see-saw bioreactor in the laboratory, Ph.D. thesis, Department of Electrical Engineering, Indian Institute of Technology, Kharagpur, India, January 1999.
[27] G. Bastin, D. Dochain, On-line Estimation and Adaptive Control of Bioreactors, Elsevier Science Publishers B.V., Amsterdam, The Netherlands, 1990.

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