

The Estimation of Electrical Equivalent Circuit Model and Behaviors of Breakdown of *Euglena sanguinea* Ehrenberg Stimulated by Multi-Level Electromagnetic Fields

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Abstract

This research studied the complex impedance and permittivities of *E. sanguinea* by measuring the RC impedance and then calculated and compared to measured values by Cole-Cole plot. The objective of this study were to build a new electrical equivalent circuit model from RC circuit model of *E. sanguinea* with MEM and explain behaviors of breakdown of *E. sanguinea* by mean of critical frequency, critical voltage, critical electric field, breakdown electric which were stimulated by MEM. The results revealed that there were a correlation between impedance from measurement and simulation with correlation value equal to 0.89. The RC impedance of *E. sanguinea* of each chemical component was behavioral in semicircle varied to frequency. The model can be used for design of wastewater treatment system by MEM such as treating algae bloom water. This is an innovative electric simulation applicable to the electric engineering and other related fields.

Keyword: Multi-Level Electromagnetic Fields (MEM), complex impedance, electric field, *Euglena sanguinea*, resonance frequency

1. Introduction

Membrane is the important chemical component of *Euglena sanguinea*. It is the external layer of cell in form of phospholipids. The organelle of *E. sanguinea* consists of protein, chlorophyll, carbohydrate, lipid and phosphorus [1]. The electrical characteristic of *E. sanguinea* cell in the form of spherical cell is shown in Figure 1A. The model of breakdown of cell is shown in Figure 1B.

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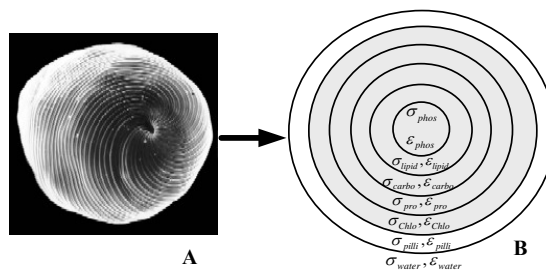


Figure 1 Surface area of *E. sanguinea* from SEM (A) and model of cell conductivity (B)

The estimation of electrical properties of each chemical component from extraction, were measured, simulated and compared by means of Cole-Cole plot technique. Moreover, the behaviors of impedance of each chemical component could be presented by a new development equation including behaviors of complex permittivity and behaviors of breakdown of *E. sanguinea* cell.

2. Materials and Methods

The water sample of *E. sanguinea* in the form of a suspended solid (1,080 mg/l) was stimulated in 10 litre (lab scale) vessel by multi-level electromagnetic field (MEM). The voltage of MEM system could be adjusted from 0 to 60 Vac and frequency was not exceeded 1 MHz as shown in Figure 2. The surface area of *E. sanguinea* and thickness were analyzed using Scanning Electron Microscope (SEM). Chemical components of *E. sanguinea* before and after being stimulated by MEM were examined by extraction and measurement of protein quantity by Bradford technique [2-3], chlorophyll by ISO 10260 [4], lipid by soxhlet [5], carbohydrate and phosphorus by digestion with acid and analyzed by spectrophotometer [6-8]. Measuring of electric current is shown in Figure 2. Resistance (R) and capacitance (C) were measured using HP 419 2 ALF Impedance Analyzer as shown in Figure 3.

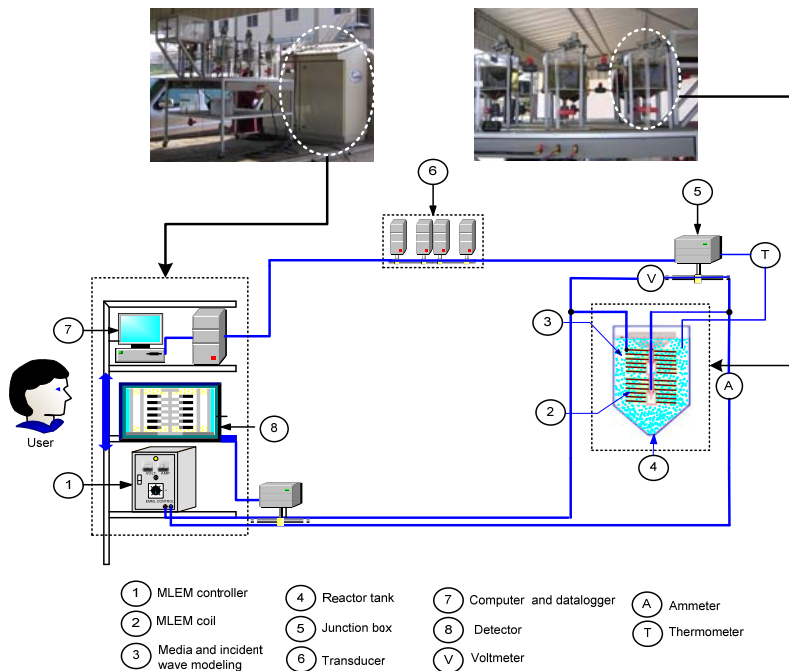


Figure 2 Experiment apparatus

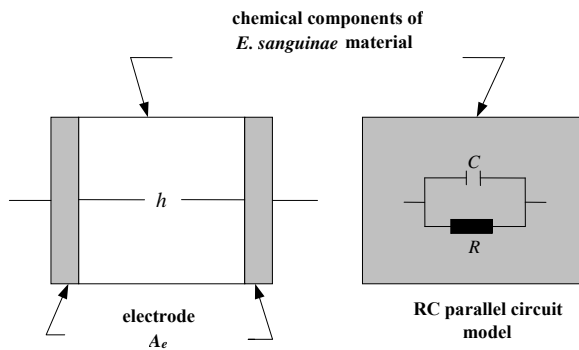


Figure 3 Diagram of RC impedance of *E. sanguinea*

3. Results and Discussion

3.1. Estimation of complex impedance of *E. sanguinea*

The results of chemical component from extraction and electrical properties are shown in Table 1. The amount of each chemical component of *E. sanguinea* from extraction, i.e., protein, chlorophyll, carbohydrate, lipid and phosphorus were 83%, 47%, 45%, 30%, and 24%, respectively. The resistance and the capacitance before and after stimulating were found to be similar.

Table 1 Concentration and electrical properties of chemical components of *E. sanguinea* before and after being stimulated by MEM

Chemical Components (com)	concentration (before)	concentration (after)	resistance (R)	capacitance (C)
protein (<i>pro</i>)	18,952 (μg/l)	3,080 (μg/l)	2.2 kΩ	0.34 nF
chlorophyll (<i>chlo</i>)	1,888 (μg/l)	1,000 (μg/l)	2.5 kΩ	0.09 nF
carbohydrate (<i>carbo</i>)	4,716 (μg/l)	2,565 (μg/l)	2.4 kΩ	0.07 nF
lipid (<i>lipid</i>)	1.126 (%)	0.785 (%)	7.9 kΩ	0.04 nF
phosphorus (<i>phos</i>)	2,567 (μg/l)	1,926 (μg/l)	4.7 kΩ	0.01 nF

The complex impedance of *E. sanguinea* was estimated by Cole-Cole plots of RC parallel circuit model. It could be represented the status of impedance which varied conformity with the frequency in the form of semi-circle. The complex impedance Z^* , real part Z' and imaginary part Z'' [9] of each chemical component were calculated using equations (1), (2), and (3), respectively. The complex impedance of cell Z^* was calculated using equation (4), real part Z' and imaginary part Z'' [9] of cell were calculated using equations (5) and (6), respectively.

$$Z_{com}^* = Z'_{com} - jZ''_{com} \quad (1)$$

$$Z'_{com} = \frac{R_{com}}{1 + \omega^2 R_{com}^2 C_{com}^2} \quad (2)$$

$$Z''_{com} = \frac{\omega R_{com}^2 C_{com}}{1 + \omega^2 R_{com}^2 C_{com}^2} \quad (3)$$

$$Z_{cell}^* = Z'_{cell} - jZ''_{cell} \quad (4)$$

$$\begin{aligned} Z'_{cell} = & 2 \left(\frac{R_{pro}}{1 + \omega^2 R_{pro}^2 C_{pro}^2} \right) + \left(\frac{R_{chlo}}{1 + \omega^2 R_{chlo}^2 C_{chlo}^2} \right) \\ & + \left(\frac{R_{carbo}}{1 + \omega^2 R_{carbo}^2 C_{carbo}^2} \right) + \left(\frac{R_{lipid}}{1 + \omega^2 R_{lipid}^2 C_{lipid}^2} \right) \\ & + \left(\frac{R_{phos}}{1 + \omega^2 R_{phos}^2 C_{phos}^2} \right) \end{aligned} \quad (5)$$

$$\begin{aligned} Z''_{cell} = & 2 \left(\frac{\omega R_{pro}^2 C_{pro}}{1 + \omega^2 R_{pro}^2 C_{pro}^2} \right) + \left(\frac{\omega R_{chlo}^2 C_{chlo}}{1 + \omega^2 R_{chlo}^2 C_{chlo}^2} \right) \\ & + \left(\frac{\omega R_{carbo}^2 C_{carbo}}{1 + \omega^2 R_{carbo}^2 C_{carbo}^2} \right) + \left(\frac{\omega R_{lipid}^2 C_{lipid}}{1 + \omega^2 R_{lipid}^2 C_{lipid}^2} \right) \\ & + \left(\frac{\omega R_{phos}^2 C_{phos}}{1 + \omega^2 R_{phos}^2 C_{phos}^2} \right) \end{aligned} \quad (6)$$

The estimation of complex impedance (Z^*) was related to complex permittivity (ϵ^*) as shown in equations (7) and (8) [10], respectively.

$$\epsilon_{com}^* = \epsilon'_{com} - j\epsilon''_{com} = \frac{1}{j\omega C_0 Z^*} \quad (7)$$

$$\epsilon'_{com} = \frac{Z''}{\omega C_0 Z^2}, \epsilon''_{com} = \frac{Z'}{\omega C_0 Z^2}, C_0 = \epsilon_0 \frac{A_m}{l} \quad (8)$$

Where ϵ' , ϵ'' and ϵ_0 are real part, imaginary part of permittivity and permittivity of free space, respectively, A_m is the end area (7.49×10^{-4}) m and l is the thickness of sample (4×10^{-5}) m (Figure 4), Z is impedance modulus ($\sqrt{Z'^2 + Z''^2}$) and C_0 is capacitance of vacuum.

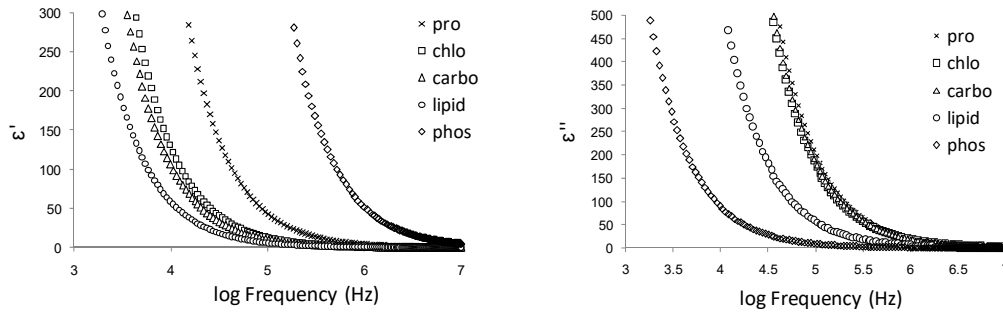


Figure 4 Real part ϵ' and imaginary part ϵ'' of each chemical component of *E. sanguinea* cell.

Behaviors of real part and imaginary part of permittivity varied according to frequency (Figure 4). Real part and imaginary part could be observed at high frequency but the value decreased. In addition, the permittivities should increase if the frequency is less than 2 MHz [11].

The capacitance (C) [10] and resistance (R) [12] of chemical component were calculated using equations (9) and (12), respectively. Equations (1) to (6) were used to calculate the impedance value and then compared with the measured value.

$$C_{com} = \frac{\epsilon_0 \epsilon_{r(com)} A}{\delta} \dots F \quad (9)$$

Where C_{com} is capacitance (C) of each chemical component of cell (F), $\epsilon_{r(com)}$ is relative permittivity of each chemical component of cell at 10 MHz, A is surface area of cell from SEM (5.02×10^{-9} m²) and δ is thickness of cell from SEM (70 nm). The diffusion permeability coefficient $P_{D_{com}}$ was calculated using equation (10) [12].

$$P_{D_{Bio-com}} = \frac{[before]_{Bio-com} Vol}{[after]_{Bio-com} \Delta t} cm^2 s^{-1} \quad (10)$$

Where $P_{D_{com}}$ is diffusion permeability coefficient of each chemical component of cell, $[before]_{com}$ and $[after]_{com}$ is concentration of each chemical component from extraction before and after stimulated with MEM as shown in Table 1 and, Vol_{cell} is volume of *E. sanguinea* from SEM ($3.51 \times 10^{-10} cm^3$) and Δt is retention time (600 sec) [11]. The conductivity [13] was calculated using equation (12).

$$G_{com} = \frac{F^2 z^2 P_{D_{com}} [a_{com}]}{\bar{R} T} \Omega^{-1} cm^{-1} \quad (11)$$

Where G_{com} is conductivity of each chemical component of *E. sanguinea* ($\Omega^{-1} cm^{-1}$), $[a_{com}]$ is concentration of each chemical component before and after stimulated with MEM (pro = 3.37×10^{-4} , chlo = 1.5×10^{-3} , carbo = 3.50×10^{-3} , lipid = 1.8×10^{-3} and phos = 8.0×10^{-4}) (mol/l), z is the number of valence electrons (pro = 22, chlo = 18, carbo = 11, lipid = 11 and phos = 21), \bar{R} is gas constant ($J mol^{-1} K^{-1}$) and T is temperature (K). The resistance (R) could be calculated using equation (12).

$$R_{com} = \frac{\delta}{\sigma_{com} A} \Omega \quad (12)$$

The inducement of voltage coil in MEM was calculated using equation (13) [14].

$$L_{coil} = \left(\frac{\mu n^2 \pi r_{coil}^2}{l} \right) \left\{ \sqrt{1 + \left(\frac{r}{l} \right)^2} - \frac{r}{l} \right\} ... H \quad (13)$$

$$XL_{coil} = 2\pi f L_{coil}$$

Where L_{coil} is inducement of MEM (1.58 H), XL_{coil} is reactance of coil and μ is permeability of copper (1), n is number of cycle of voltage coil (5.25), r_{coil} is the radian of voltage coil (6 mm.), l is the length of voltage coil (1950 mm.), f is frequency (Hz) and resistivity R_{coil} equal to 50Ω , the electrical equivalent circuit model could be developed as shown in Figure 5.

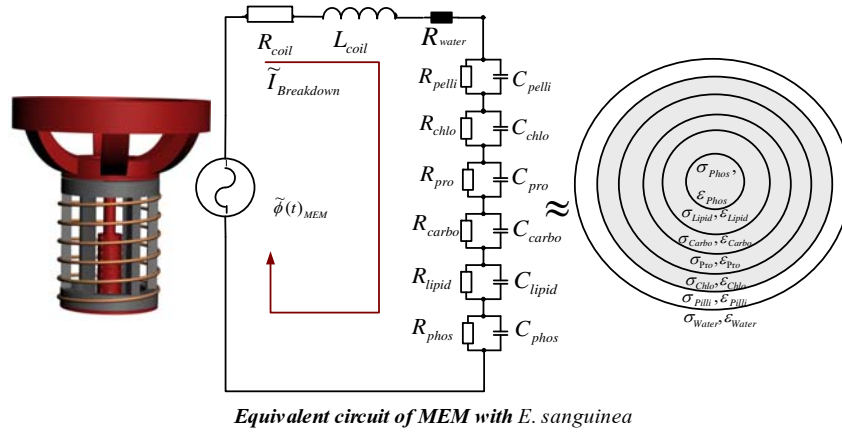


Figure 5 Equivalent circuit of MEM with *E. sanguinea*.

Figure 6 shows semi-circle graphs from measurement at frequency less than 1 MHz. It is in agreement with the result of Pan *et al.* [9]. The impedance in form of real part Z' and imaginary part Z'' from measurement and simulation with correlation equal to 0.89 at 95% confidence interval. These graphs were dropped at high frequency as its value decreased with the increase of frequency. Electrical equivalent circuit model could reduce the complexity of *E. sanguinea* with MEM which was developed in order to facilitate MEM system design and explain many phenomena in cell. In addition, these were the information for calculating and designing the MEM system.

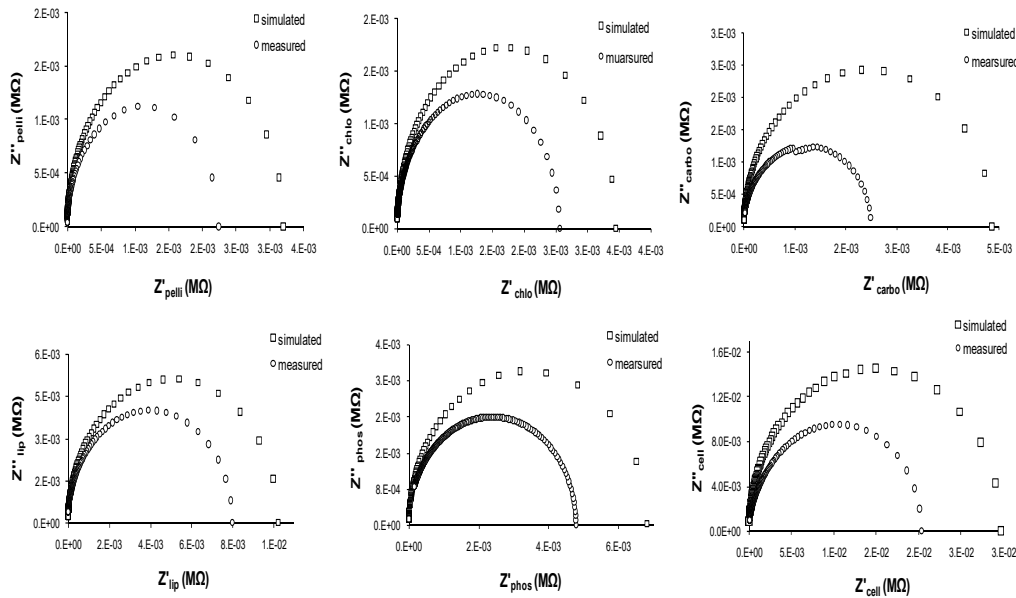


Figure 6 Cole-Cole plots from calculation and measurement of chemical component from each part of cell and cell of *E. sanguinea*.

3.2. Resonance frequency of each chemical component of *E. sanguinea* cell

The electrical equivalent circuit model was stimulated by AC signal. The condition of resonance was made by the same electrical circuit as shown in Figure 7. The researcher used resonance frequency to explain many phenomena of breakdown in each chemical component of *E. sanguinea* cell. Moreover, good capacitance let electrical power not distribute but generate the energy in form of real capacitor because effect of solenoid electrode. Therefore, the loss of capacitance and resistance was depending on factor in equation (15).

$$R = \frac{1}{\omega C \tan \delta} \quad (14)$$

$$\tan \delta = \frac{1}{\omega C R} \quad (15)$$

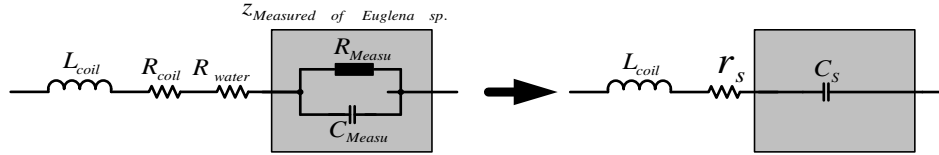


Figure 7 Equivalent circuit of complex impedance of *E. sanguinea* with impedance of coil for simulated resonance frequency.

Changing figure from left to right C_s could be calculated by equation (16).

$$C_s = \frac{C + \frac{1}{R^2 \omega^2 C}}{1 - \frac{L_{coil}}{R^2 C} - \omega^2 L_{coil} C} \quad (16)$$

Where ω is angular frequency.

$$r_s = R_{coil} + R_{water} + \frac{\tan \delta}{\omega C_s} \quad (17)$$

$$|Z| = \left[r_s^2 + \left(\omega L_{coil} - \frac{1}{\omega C_s} \right)^2 \right]^{\frac{1}{2}} \quad (18)$$

Resistance, reactance or impedance was value of electrical conductivity in AC circuit but X_C and X_L depended upon frequency of energy sources. It could be observed that the less Z value was the more total electricity in circuit was. In case of AC circuit, the least Z value occurred when $X_C - X_L = 0$. It would be called “resonance frequency” if it was in the form of angular frequency and in this condition total electricity in the circuit would be the highest.

The results of resonance frequency in form of resistance (r_s) and impedance (Z) from measurement and simulation of each chemical component are shown in Figures 8-12. The resistance value was relatively stable. The impedance varied according to curvature and if impedance value decreased, resonance frequency would decrease. The resonance frequency of protein, chlorophyll, carbohydrate, lipid and phosphorus from measurement were 2×10^4 , 2.3×10^4 , 2.5×10^4 , 2.2×10^4 , and 3×10^4 Hz, respectively.

Figure 5 depicts the equivalent circuit. Cell of *E. sanguinea* was broken down by electric transmitting through total cell. It always initially makes cell membrane or cell wall changed. Therefore, it should be focus on this point and the researcher paid attention especially for membrane or it was so called phospholipids which were lipid and phosphorus [12] to explain the breakdown of cell by assuming that there are less values of the impedance of chemical component in organelle. Therefore, in this study, the model [9] as shown in Figure 13 was selected. The solutions from equations (19) and (20) were shown in Figure 14.

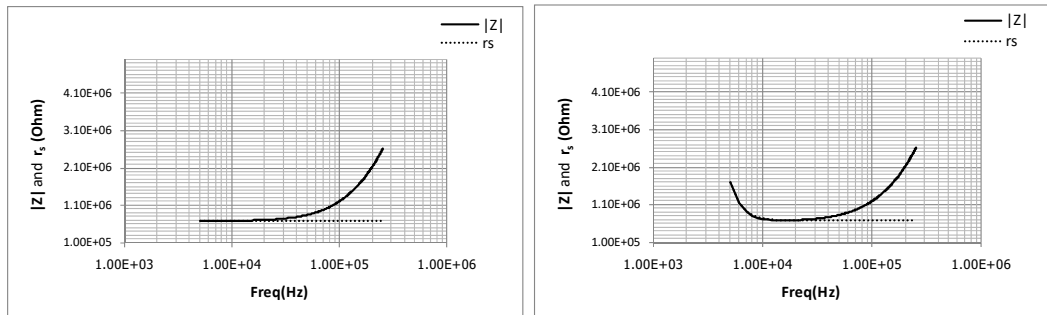


Figure 8 Response of the equivalent series resistance r_s and the impedance $|Z|$ of protein: left: measured, right: simulated

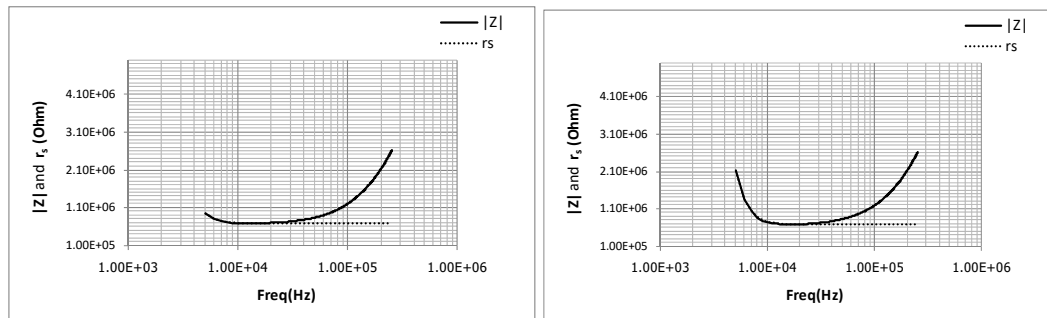


Figure 9 Response of the equivalent series resistance r_s and the impedance $|Z|$ of chlorophyll: left: measured, right: simulated

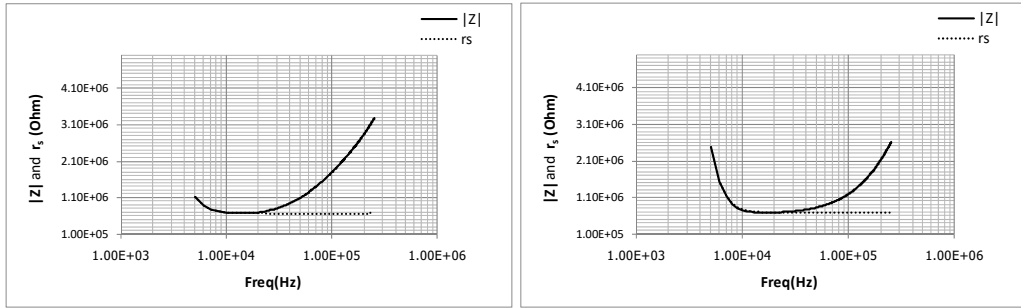


Figure 10 Response of the equivalent series resistance r_s and the impedance $|Z|$ of carbohydrate; left: measured, right: simulated

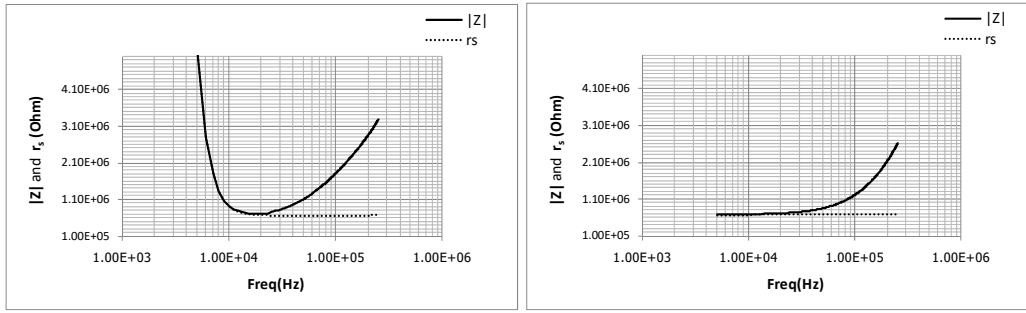


Figure 11 Response of the equivalent series resistance r_s and the impedance $|Z|$ of lipid; left: measured, right: simulated

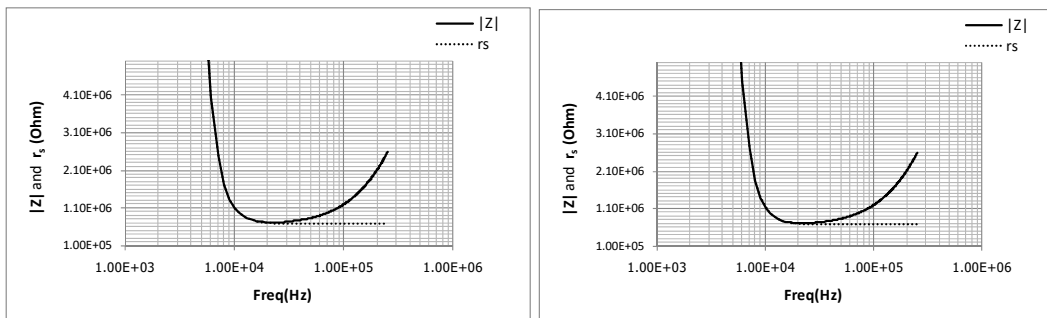


Figure 12 Response of the equivalent series resistance r_s and the impedance $|Z|$ of phosphorus; left: measured, right: simulated

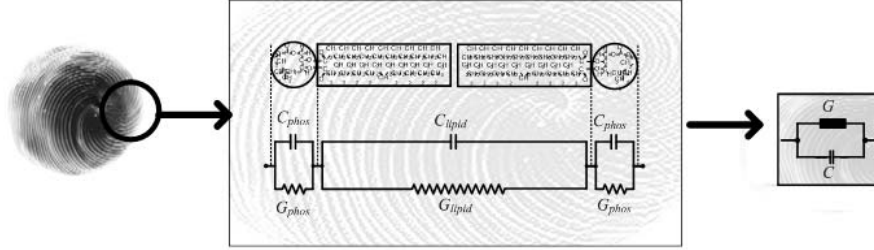


Figure 13 RC circuit model of membrane.

$$C = \frac{C_{lipid} G_{phos}^2 + 2C_{phos} G_{lipid}^2 + \omega^2 (C_{lipid} C_{phos}^2 + 2C_{lipid}^2 G_{phos})}{(G_{phos} + 2G_{lipid})^2 + (\omega C_{phos} + 2\omega C_{lipid})^2} \quad (19)$$

$$G = \frac{G_{lipid} G_{phos}^2 + 2G_{lipid}^2 G_{phos} + \omega^2 (C_{lipid} C_{phos}^2 + 2C_{lipid}^2 C_{phos})}{(G_{phos} + 2G_{lipid})^2 + (\omega C_{phos} + 2\omega C_{lipid})^2} \quad (20)$$

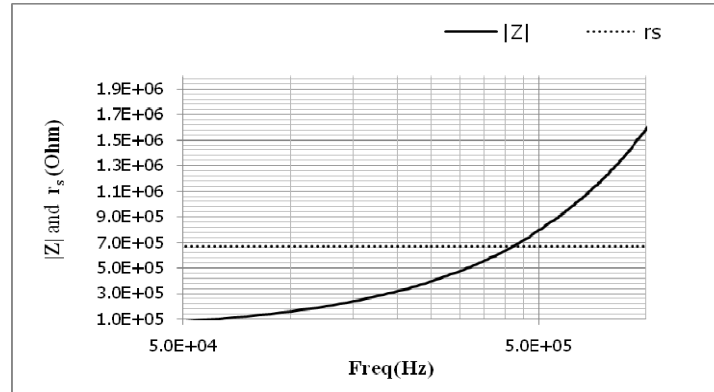


Figure 14 Response of the equivalent series resistance r_s and the impedance $|Z|$ of cell.

Figure 14 shows response of equivalent series in the form of resistance (r_s) and impedance (Z) of *E. sanguinea* cell from measurement. The reaction in membrane showed that resonance frequency was 4×10^5 Hz which was higher when compare with resonance frequency of each chemical component of *E. sanguinea* cell.

4. Behaviors of Breakdown of *E. sanguinea*

Critical voltage (V_{crit}) of *E. sanguinea* cell is given by Goster and Zimmermann [15] as shown in equation (21).

$$V_{crit} = \left[\frac{0.3679Yd_o^2}{\epsilon_o \epsilon_r} \right]^{\frac{1}{2}} \quad (21)$$

Where Y elastic modulus of *E. sanguinea* cell derived from the electric stress per unit area was calculated in equation (22), d is thickness of cell which response to electric (7 nm) and d_o is thickness of cell which does not response to electric or lipid bilayer (2mm) [16].

$$\frac{\epsilon_r \epsilon_o V_m^2}{2d^2} = Y \ln \frac{d_o}{d} \quad (22)$$

The critical voltage (V_{crit}) is related to electric field (E_{crit}) from equation (23) [17] where N_B is concentration of *E. sanguinea* cell from Table 1.

$$V_{crit} = \frac{\epsilon_s E_{crit}^2}{2eN_B} \quad (23)$$

Where ϵ_s is permittivity of *E. sanguinea*. Replaced equation (21) into equation (23) to obtain equation (24).

$$E_{crit} = \sqrt{\frac{2e}{N_B \epsilon_s} \left[\frac{0.3679Yd_o^2}{\epsilon_o \epsilon_r} \right]^{\frac{1}{2}}} \quad (24)$$

Figure 15 shows that the behavior of critical electric field varied according to thickness of membrane. The electric field will increase if thickness of membrane increases. However, critical electric field varied indirectly to concentration of *E. sanguinea* cell and dielectric and permittivity varied directly to elastic modulus.

The appropriate equation of breakdown electric of *E. sanguinea* cell from Figure 2, temperature was controlled from 37°C to 45°C and stimulated voltage from 0 to 60 V. It was found that appropriated breakdown electric of *E. sanguinea* cell was power of polynomial type which $r^2=0.857$, at confident interval 95%. To obtain accurate results, equation was used in form of curve. Breakdown electric could be calculated from equation (24) using statistic data from Figures 16 and 17.

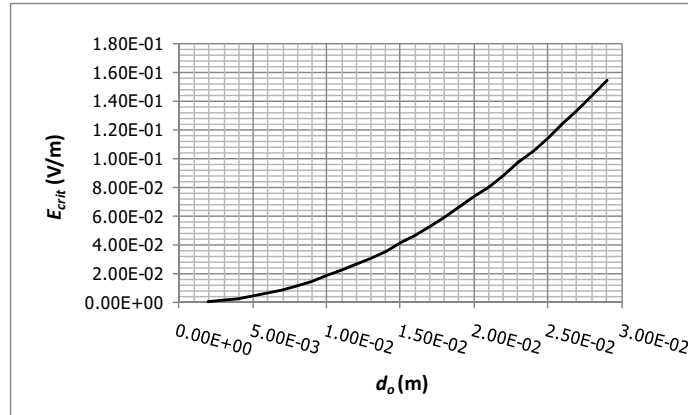


Figure 15 Behavior of critical electric field varied according to thickness of membrane

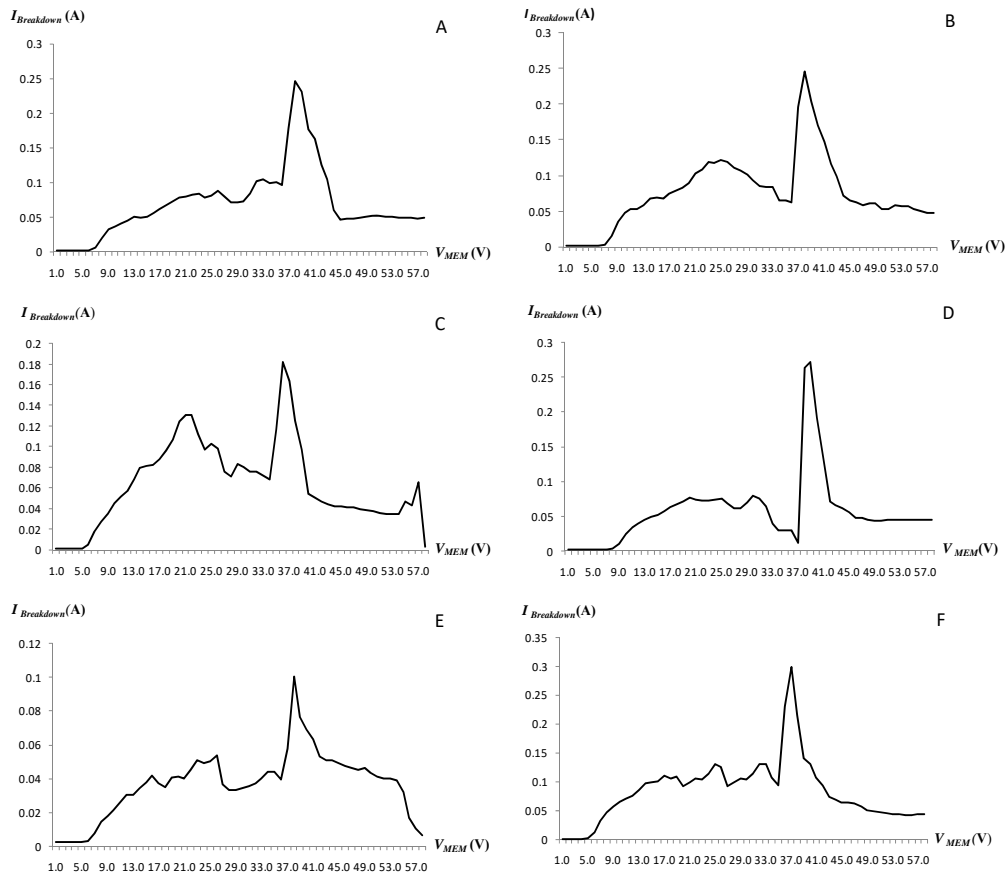


Figure 16 Behaviors of electric and impulse voltage of MEM from 6 measurements.

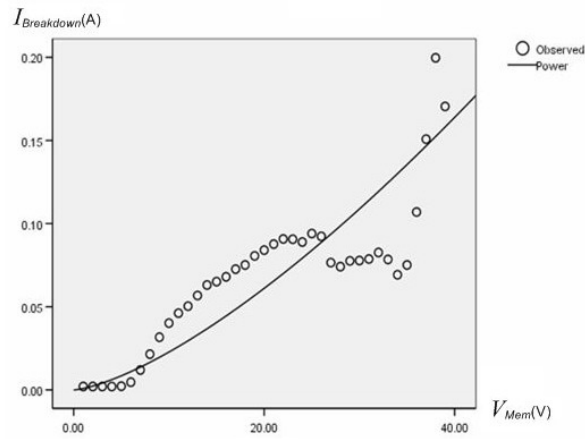


Figure 17 Breakdown electric of *E. sanguinea* cell and impulse voltage from measurement.

$$I_{Breakdown} = 0.001 V_{MEM}^{1.424} \quad (25)$$

It was found that critical voltage calculated from equation (21) equal to 5 mV. In addition, from the experiment maximum voltage (V_{MEM}) used for stimulating *E. sanguinea* cell was 60 V and it was used in equation (24). The results showed that breakdown electrical depended on impulse voltage of MEM and breakdown electrical equal to 0.34 A.

4. Conclusions

The results demonstrated that electrical properties, i.e., resistance (R), capacitance (C) and impedance before and after stimulating by MEM were very similar as it was able to maintain its specified material property. The appropriated relative permittivity could be used for designing MEM system with high frequency from 1 kHz and higher. The electrical equivalent circuit model could be applied to estimate and design MEM system for waste water treatment. Moreover, it was explained that resonance frequency was the frequency that let the maximum electric current transmitted through each chemical component of *E. sanguinea*. Critical electric field varied according to thickness of membrane and breakdown electric varied according to maximum impulse voltage of MEM. In addition, the behavior of critical electrical and critical electric field would be useful for studying the impedance of small animals and other related fields.

5. Acknowledgements

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