

SENSITIVITY OF THE ABSORBANCE AND CAPACITANCE METHODS IN MEASUREMENT THE NUMBER OF CELLS IN-VIVO

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Abstract: Have been carried out measurement of the number of cells in vivo by using two methods, namely measuring the absorbance using spectrophotometer and the capacitance of the liquid capacitor is designed himself. The results of both experiments suggests that the capacitance method is more sensitive and transparent than the absorbance method at once proves that it could only be used for a limited range of cell numbers. In contrast, the capacitance method could be used for more wider range of cell numbers.

Keywords: *E. Coli*, *Saccaromyces cerevisiae*, *Streptococcus agalactiae*, and *Aspergillus niger*, absorbance, capacitance

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I. INTRODUCTION

Usually microbiologists perform the measurement of the number of cells using absorption spectrophotometer method combined with the Total Plate Counting (TPC). By this way they could compare the level of turbidity (absorbance) a solution containing a number of cells [18]. This study seeks to develop the measurement of the number of cells with a totally different way, i.e. using liquid capacitor. Both of these measurement methods have a very different principle altogether. If the measurement via spectrophotometer rely on the interaction of light with matter present in the solution, then through the observation of the cells capacitance measurements performed by the distributed charge in solution and on the surface of the cell itself.

This study tried to compare the extent to which the two methods has a convincing level of sensitivity and transparency, will be discussed in this paper.

A. THEORY

Absorption spectrum measurement using a spectrophotometer based on Lambert-Beer law, that the intensity of light absorbed (I) is proportional to the intensity of light source (I_0) which varies exponentially as a function of the thickness of samples (the optical path of light, d), the concentration of the solution (c) and extinction coefficient (ϵ). If written in the absorbance (A):

$$A = \log\left(\frac{I_0}{I}\right) = \epsilon d l \quad (1)$$

Extinction coefficient, ϵ , depends on the chromophore (the molecule has a dipole moment) that interact with light. Absorbance was measured for specific bacteria at 365 nm.

The larger dipole moment of the population, the greater the number of bacteria in solution, the intensity of the absorption will be greater.

In contrast to the spectrophotometer principles, the capacitance of the solution containing cells/bacteria is a reflection of the charge distribution inside the solution and containing bacteria (cells). Because the cells and also the

solution used is a dielectric materials, the charge arising in it are induced charge, which are derived by electric field between capacitor plates. Therefore, as the first approximation, the value of the total capacitance (C_{total}) of the capacitor used is the algebraic sum of the value capacitance of the solution (C_{Solution}) and capacitance of the cells (C_{cell}):

$$C_{\text{Total}} \approx C_{\text{Solution}} + C_{\text{cell}} \quad (2)$$

In accordance with the theory of cell growth during the time t , the cells develop according to the equation:

$$N_t = N_0 e^{\lambda t} \quad (3)$$

N_t , N_0 respectively is number of cells in time t and in $t = 0$, λ is the growth constant of the cell.

II. MATERIALS AND METHODS

To measure the capacitance of the cells in-vivo by using a capacitor, first one must be made the capacitor by his-own, making it possible to measure the capacitance of the solution. Then this capacitor should be tested by using a known material, it means that the capacitance values of this materials is also known (in this experiment one used distilled water).

- Equipment used in the experiment is as follows:
 - Parallel plate capacitor
 - Capacitance measuring instrument in the form of Dual Display LCR-meter ELC-131D serial number 40,224,023
 - UV-VIS Spectrophotometer Variant 2415
 - Incubator Ogawa Seiki Co., LTD.
 - Waterbath thermologic NVC
 - Autoclave DSK 6508
 - Freezer
 - Maxi Mix II, thermolyne / Barnstead type 37 600 no.3850580
 - Stirrer, thermolyne / 2555 Kerper Boulevard Dubugue Barnstead, Iowa no.757960475604

– General microbiology laboratory equipment.

▪ Materials:

- E. coli pure culture (UICC B-14)
- S. Agalactiae (former veterinary IPB)
- S. Aureus (former veterinary IPB)
- Bacterial Cultur Medium (TEB / TEA)
- Sodium chloride (NaCl)
- Distilled water
- Alcohol, peptone, malt extract, yeast extract, glucose, in order

▪ Measurement procedures:

Measurements were performed by using capacitors as follow:

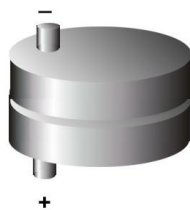


Figure 1. Diagram of liquid Capacitor

In general, experiments were carried out in room temperature.

III. RESULTS AND DISCUSSION

A. Calibration capacitor and Capacitance of distilled water

It is important to check performance of capacitor, before it's being used to measure capacitance of cells. The capacitor have been tested by using distilled water which we known its capacitance value. Capacitance value of distilled water which measured was 76.3 pF (at room temperature), while in the literature, its values was 77.29 pF (at room temperature). So that the relative error to the literature range about ~ 2%.

Because existence of non-polar molecules in the medium on one side and the Brownian motion on the other hand, the values of liquid capacitance in a moment would be unstable, it would fluctuate for a while. By administering electric field in medium, dipole moment gradually polarized, up to the direction of given electric field (see figure 3).

B. Capacitance and Absorbance of Bacteria E.coli

Dominant component of the macromolecular constituent of cells are dipolar molecules, such as H₂O, CO₂, H₃- etc [19]. Without an electric field, these molecules have permanent dipole moments with random orientation. By administering an electric field, dipole moments will be oriented according to the direction of the field. Influence of the electric field is then created excess charge which may be

positive or negative on each surface of the medium as a whole. These induced charges could be distributed at the cell surface and medium. Capacitance values of bacteria in vivo could not be obtained directly, but measurements were made for the bacteria that live in the solution. By knowing the mixed capacitance of live bacteria and its medium, and the capacitance of medium itself, can be calculated the capacitance of bacteria. Capacitance measurement procedure performed bacteria-medium by measuring the variation of capacitance values over the time. As mention above, the growth of cells depend exponentially with time.

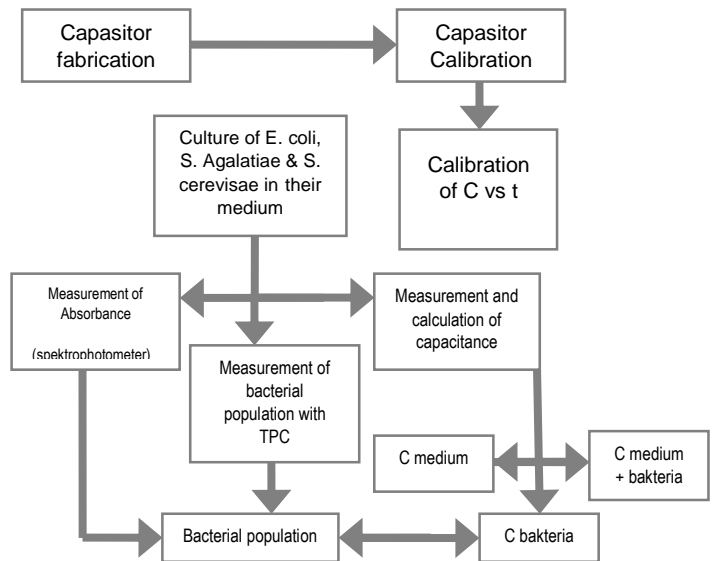


Figure 2. Measurement diagram and calculation procedures of the cell capacitance and the absorbance measurements of the E. coli in solution

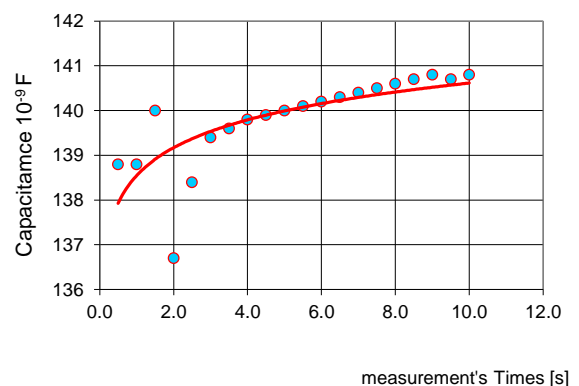


Figure 3. Variation of capacitance values with respect to time. At the time of the arrangement induced dipole moment corresponds to the direction of the electric field is given, will be obtained the values of capacitance constant. This can be evidenced by Figure 2, the values of distilled water capacitance, $C = C(t)$.

Figure 4 is illustrated the growth of cells during times. These curve also proves that the growth of cells is exponentially proportional throughout the time.

From the curve of cell growth can be concluded that the cell population is proportional to the values of the capacitance (see figure 3), or:

$$C \text{ (Capacitance)} \propto N \text{ (number of cells)}$$

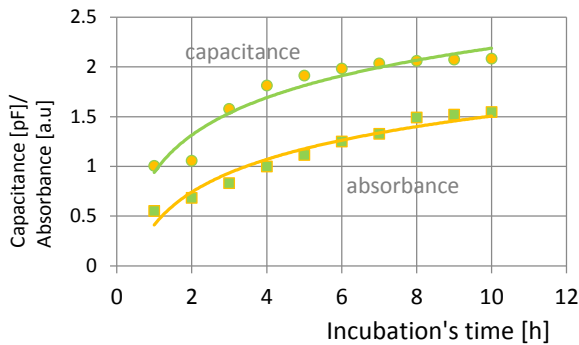


Figure 4. Sample evidence of capacitance of E.coli versus incubation's times.

With the increasing growth of bacteria over time, the values of the capacitance also increases, it means that each bacterium and also medium must contribute to the overall values of the capacitance. In assumption that the number of cell capacitance is proportional to the number of cells, it can be proved directly in Figure 4. This figure also shows the comparison of bacterial growth of *E. coli* of incubation time or the number of bacteria that illustrated in cell volume. This means that the volume of each cell is identical to the number of cells.

Figure 5 illustrated that the capacitance of cells is proportional directly to the absorbance and also to the number of cells, if one describe the absorbance, capacitance as a function of number of cells.

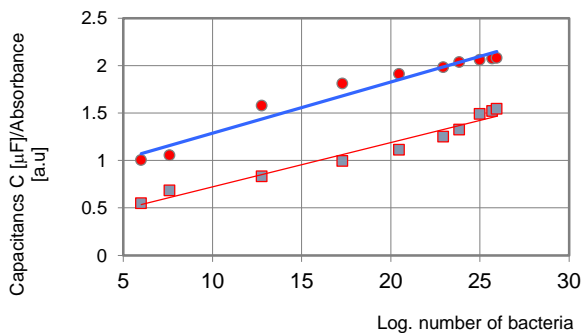


Figure 5. Relation between capacitance of and number of E. coli has linear correlation.

C. The relation of Absorbance, Capacitances, and Number of Bacteria

Absorbance measurements on the growth of *E. coli* bacteria was carried out up to 24 hours. Together prior to the measurement of absorbance (at wavelength 365 nm) was measured capacitance of each clip (see also figure 4). Capacitance and absorbance curves shows the consistent evidence, that both curves, respectively proportional to the number of bacteria in solution.

As shown in figure 5, figure 6 illustrates unnormalized and normalized curves of capacitance and absorbance. The capacitance curve is always higher than the absorbance. This fact indicates that the capacitance is more transparent or sensitive to the number of bacteria in solution compared to the absorbance. It is clear that as the basic parameter of capacitance is charge, while relying on the light absorbance, the more concentrated solution, the ability of equipment to record the light absorption is limited.

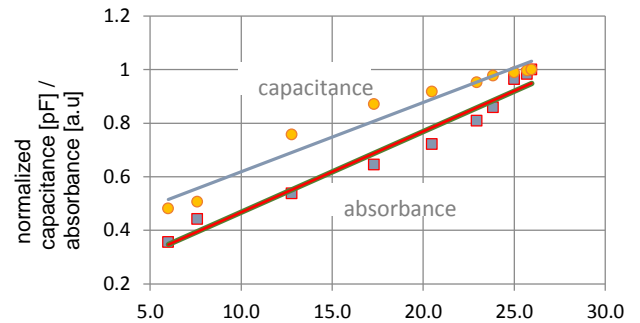


Figure 6. Comparition of normalized absorbances and Capacitances of E. coli cells. The differences between two curves shows the sensitivity of the metods.

This fact shows that the values of the capacitance is proportional to the values of absorbance and also proportional to the bacterial populations:

$$C \text{ (Capacitance)} \propto N \text{ (number of cells)} \propto A \text{ (Absorbance)}$$

For proving quantitatively, the variation of capacitance and the absorbance curves of *E. coli* in variation logarithmic number of cells were illustrated in figure 6. Both curves show the consistency of the increasing number of cells and also are proportional to the number of bacteria.

The interesting thing to note is, there are significant difference sensitivity respons of the number of cells/ bacteria through absorbance and capacitance measurements. The number of bacteria more sensitive by using a capacitor than by spectrophotometer (absorbance). If the relative sensitivity between capacitance and absorbance methods is defined as $\Delta S = (C-A) / C \times 100\%$, ΔS is about 45% (relative to the capacitance values) if the number of bacteria present in the solution ranging from 10^6 to 10^{17} . If the number of bacteria more than 10^{17} such differences (sensitivity) begin to decrease. This is understandable due to reabsorption of light when the number of bacteria present in the solution is greater than 10^{17} . The greater the number of bacteria up to 10^{17} to 10^{25} , the sensitivity was decreased, namely from 37% to 25%. This methods have been proved for different bacteria, such as *Saccaromyces cerevisiae*, *Streptococcus agalactiae*, and *Aspergillus niger* [1,2,3].

As we known, that the principle of capacitance measurement refers to the amount of induced charge contained in the solution and the cell's surface, through the existence of electric field on both of capacitor plate. While the absorption relying of light which is absorbed by solution containing the bacteria. The more viscous or more number of cells present in the solution, the absorption of incident light

by the bacteria is inhibited due to reabsorption of light by the surface of the bacteria in question, so that the light detected at the detector isn't contains the real information of the samples.

IV. CONCLUSION

From this experiment can be concluded as follows:

- The absorbance's values, proportional to the capacitance and is proportional to the number of cells containing in solutions:

$$A \sim C \sim N$$
- Capacitors are more sensitive than absorbance in the measurements of bacteria.
- These experiments showed that the Lambert-Beer's law no longer transparent or sensitive if the number of bacteria is greater than 1017.
- Capacitors can be used to measure the amount of bacteria for more than 1025 of bacteria.

REFERENCES

- [1] Musaddiq Musbach, Iman Santosa, Maulana, Wamid Antaboga. Calculation Model of Individual Capacitance of *Aspergillus Niger* Cell based on Experimental Data. Nasional Seminar on Physics, will be held on University of Sriwijaya, Palembang, 8 June 2012 (submitted).
- [2] Musaddiq Musbach, Iman Santosa, Maulana, Wamid Antaboga. Capacitance Measurement of *Aspergillus niger* cells, South East Asia Conference on Medical Physics and Biophysics, 2010.
- [3] Musaddiq Musbach, Iman Santosa & Paul Christian. Measurement of Capacitance per Cell of *Saccaromyces cerevisiae*. Seminar on Biophysics and Medical Physics Society, RMSB 2007, IPB
- [4] Musaddiq Musbach, Ariesty M, Lilis Kurniawati, IWT Wibawan, Siswati Setiasih. Capacitance measurement of *E. Coli*, *S. Agalactiae* and *S. Aurens*. Seminar on Biophysical and Medical Physics Society, IPB, 2005.
- [5] Musaddiq Musbach et al. Determination of life *Eschericia Coli* capacitance, 2002, Seminar on Biophysical and Medical Physics Society, IPB, 2002.
- [6] Richard A, Principles of Biomedical Instrumentation and Measurement. Maxwell Macmillan Int. Pub. Comp.New York,1995.
- [7] HC Berg and L Turner, Torque Generated by the Flagellar Motor of *E.coli*. Biophysical Journal, Vol.65, Nov 1993.
- [8] Collquhoun, K.O., S Timms and C.R.Fricke, Detection *E.coli* in Potable Water using direct Impedance Technology. Journal Application Bacteriology, Vol.79:635-639, 1995.
- [9] Wibawan, IWT and C. Lammler, Relation between Serotype of Streptococci of Serological Group B and Growth Rate at Early Logarithmic Phase. Paul Parey Scientific Publishers, Berlin dan Hamburg, 1993.
- [10] K.H.Iwasa, Effect of Stress on the Membrane Capacitance of the Auditory Outer Hair Cell. Biophysical Journal, Volume 65:492-498, 1993
- [11] H.H.Willard, L. L. Merritt Jr, JA Dean,FA Seattle Jr, Instrumental Methods of Analysis. Wadsworth Publishing Comp.,Belmont, Seventh ed.1992.
- [12] Gandjar et al, Pedoman Mikrobiologi Dasar, (Biologi FMIPA UI 1992)
- [13] Gandjar et al, Pedoman Mikrobiologi Dasar, (Biologi FMIPA UI 1992)
- [14] Butterfield, Christensen, Curtis dan Gunlazuardi, Water Disinfection using an Immobilised Titanium Dioxide Film in a Photochemical Reactor with Electrofield Enhance-ment, (Water Research, 1992)
- [15] EDP De Robertis, EMP De Robertis,Jr., Cell and Molecular Biology. International Copyright Union, Eight Ed.1987.
- [16] Fricke et al, Dielectric Properties of Nitrocellulose Water Gels. Nature Journal, Vol 177: 1106-1107, 1953.
- [17] Siddal G., vacuum, 9, 274, 1959.
- [18] Fricke et al, A Dielectric Study of Low Conductance Surface Membrane in *E.coli*. Nature Journal Departement of Bacteriologi, Vol.177, 1956.
- [19] Fricke et al, Relation of the Permittivity of Biological Cell Suspension to Fractional Cell Volume. Nature Journal, Vol 172: 731-732, 1953.
- [20] Hass,G.,J.Am.Ceram.Soc.33,353,1950.