# MEASUREMENT OF ELECTRODE IMPEDANCE AND ELECTRODE CONTACT VOLTAGE FOR THE DETECTION OF MICROORGANISMS

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*Abstract: Rapid detection of microorganisms in liquid samples by means of electric impedance measurements has become a world-wide applied hygienic standard method. A so-called raster electrode method, specifically adapted for measurements on microorganism cultures, yielded effective analyses of dielectric properties providing information on both the dielectric properties of the liquid culture medium and the properties of the inserted electrodes. Without the need of reference measurements of electrode characteristics, the method allows for the low frequency determination of a cell culture's conductivity $s$ and permittivity $e$ as a function of cell concentration n. For routine monitoring of long-term growth processes, the novel method takes advantage of $s$ but also of time changes of the boundary layer's electric impedance and contact voltage. Accelerated detections are striven for by a pre-concentration of cells by dielectrophoresis prior to the impedance measurement. The new methods prove to be effective tools for the detection of different types of bacteria and yeast which show high relevance in food technology or medicine.*

*Keywords: bio-impedance measurement, electrode impedance, contact voltage, biomedical diagnostics*

## 1   INTRODUCTION

Rapid detection of microorganisms in liquid samples by means of electric impedance measurements has become a world-wide applied hygienic standard method. Methods have been reported for the detection and quantification of bacteria, e.g., in meat [1], milk and milk products [2,3], fish [4] and vegetables [5]. These impedimetric measurements are also used for the detection of yeasts and moulds [6]. Most instruments measure the modulus $Z$ of the complex impedance, often expressed as "resistance", or the inverse value "conductance".

For the determination of microorganisms, the samples are suspended in liquid nutrient media and transferred into measuring tubes containing an electrode system for impedance measurement. During the incubation time, the measured value of $Z$ changes in case of presence of microorganisms. Due to the microbial metabolic activity, nutrients are converted into smaller charged components. The latter show increasing contribution to the transport of current, thereby decreasing $Z$. In some cases, also the contrary results - uptake of ions into the cell or chemical /enzymatical reactions yield increases of $Z$.

However, $Z$ shows restricted applicability with respect to selective detection of microorganism groups or species. Here, selective culture media are necessary which need specific additives [7]. The latter tend to exhibit ionic structure, thus increasing the initial ionic content of the nutrient broth. As a consequence, ions produced cannot be detected by measuring $Z$. As well, $Z$ fails to indicate the existence of yeasts and moulds due to their low metabolic activity.

On the other hand, impedance or capacitance measurements are used in fields of biotechnology for on-line control of fermenting processes [8]. In this case, the a priori concentration of microorganisms is high enough to ensure *direct* influences on the measured electric quantities. Dielectric properties of cell suspensions have been analyzed in numerous studies, providing information on cellular characteristics, membrane characteristics and also on the composition of suspension media. Extensive investigations have been focused on properties of blood. Its conductivity $s$ depends strongly on the chemical composition of the plasma [9] and shows strong decreases with increasing concentration $n$ of erythrocyte cells [10,11]. This offers a rapid determination of the haematocrit, the reliability of results however being restricted by the fact that $s$ is also proportional to the ionic content of the plasma which depends on the type of blood. Also, the permittivity $e$ of blood has been determined. Measurements performed

at a frequency of 50 kHz by means of the here-discussed raster electrode technique [10] have shown increases of $e$ with increasing $n$, $e$ reaching a maximum for haematocrit values close to 70%, followed by decreases for even higher concentrations. Maximum values for $e$ increased with increasing cell size $d$ (diameter), being close to 2000 for sheep blood ($d \approx 5$ µm) and 10000 ($d \approx 10$ µm) for elephant blood. Permittivities of similar order of magnitude have been determined for suspensions of tumor cells, values of only some thousand resulting in spite of rather large $d$ ($\approx 15$ µm) due to low $n$ [12]. Measurements on unicellular microorganisms [13-15] like yeasts ($d \approx 3$ µm) and bacteria ($d \approx 1$ µm) yielded permittivities of the magnitude of 900 and 500, respectively.

Especially in cases of small cell size, analyses are complicated by electrode polarization effects, the electrodes representing a complex impedance $\underline{Z}_E$ which depends on the frequency $f$ and on the current density $S$ among others. As well known, $\underline{Z}_E$ shows a distinctly capacitive behavior, while the impedance $\underline{Z}_S$ of the suspension tends to show a very small capacitive component - Im ($\underline{Z}_S$) (Fig. 1). The low ratio $r$ = Im( $\underline{Z}_S$) / Im( $\underline{Z}_E$) yields the main problem for effective determinations of the permittivity $e$ of the suspension. Apart from coating platinum electrodes with platinum black to reduce electrode polarization [13], many authors apply the well known four-electrode technique in order to suppress polarization effects [15]. However, small particles tend to yield extremely low values of $e$ and thus also a very low ratio $r$. As a way out of the dilemma, reference [8] reports the possibility to determine a "polarization control" for $n = 0$ and to subtract this value from the equivalent cell data assuming that $\underline{Z}_E$ is constant for all measurements. As demonstrated by the results of the present paper, $\underline{Z}_E$ changes considerably, e.g., during the microorganism growth which indicates respective drawbacks of the subtraction technique.
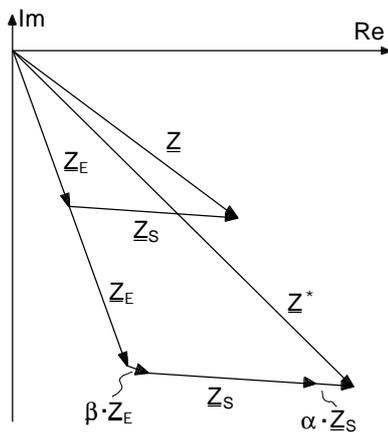


**Figure 1.** Vector diagram of impedance components (qualitative outline). Sum impedance $\underline{Z}$ and $\underline{Z}^*$ for both raster segments active and one raster segment active, respectively (see text).

Also for the separation of the measured sum impedance $\underline{Z}$, several approaches have been reported in literature. Reference [16] reports a frequency variation, subsequent extrapolation yielding the low-frequency values in approximation. Alternatively, in [17,18] the real component of $\underline{Z}$ is attributed to the suspension and the imaginary component to the electrode, a procedure which however dispenses with the determination of capacitive components a priori.

An other possibility, which has been reported to determine the two impedance portions $\underline{Z}_S$ and $\underline{Z}_E$ for a given (low) frequency value $f$ uses two measurements performed with different distances of electrodes [19]. However, it is based on an identical value $\underline{Z}_E$ for both measurements, a condition which may be invalidated even by minute differences of gas bubble concentrations at electrode surfaces. For us, this distance variation technique has been a stimulus to develop the here-discussed raster electrode technique which is based on a variation of the effective electrode area. Earlier, we have designed this method for analyses of blood cell suspensions [10]. It enables synchronous determinations of the complex impedance $\underline{Z}_S$ of the cell suspension and the complex impedance $\underline{Z}_E$ of the electrode system for both a given frequency and a given point of time - two measurements being performed within a second.

The present paper is focused on those fields of electrical analyses of microorganism cultures in which effects of electrode polarization play an important role: (i) the characterization of highly concentrated cells by means of dielectric quantities, (ii) the characterization of growth processes by measurements of the electrode impedance and the electrode contact voltage, and (iii) a pre-concentration of cells by means of dielectrophoresis prior to the impedance measurement.

While the first field represents a "classical" discipline, the second gained practical significance in recent time. An increasing amount of papers discusses time changes of the conductivity $s$ arising

during growth processes. Respective measurements have become a routine tool for contamination checks in different fields of hygienics [1-7] in spite of the fact that the origin of the measured quantities has not been fully clarified. The here discussed raster electrode proves to be an effective tool for corresponding clarifications as it offers knowledge about metabolism-caused changes of electrode characteristics. As demonstrated by experience [16,25], also these characteristics can be utilized for routine checks of contamination. Finally, the automatic pre-concentration and subsequent detection through $\underline{Z}_S$ or $\underline{Z}_E$ promises a novel procedure for an almost spontaneous detection of low microorganism concentrations in various fields of application.

## 2    PRINCIPLE OF MEASUREMENTS

The basic principle of the above mentioned raster electrode technique has been described in detail in [10]. Thus, the following is restricted (i) to the broad features of the method and (ii) to specific modifications which proved to be necessary for effective applications on microorganisms.
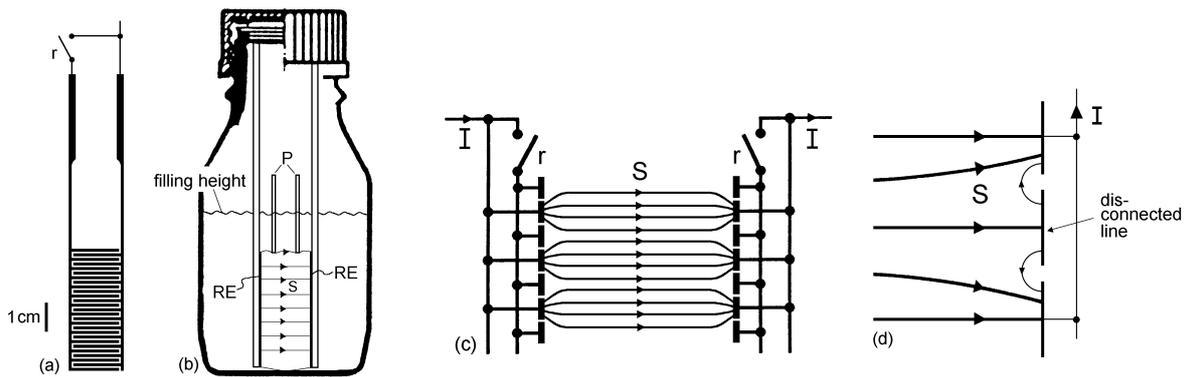


**Figure 2.** Outline of the raster electrode technique.

(a) Example for the design of a raster electrode. Opening of the switch r yields approximate doubling of the effective electrode impedance.
(b) Example for the arrangement of raster electrodes (RE) in a culture tube.
(c) Schematic outline of flux distribution in the case of disconnected raster halves.
(d) Detail of (c) illustrating the bridging function of disconnected lines.

The method is based on the assumption that the (measured) over-all impedance $\underline{Z}$ of an (electrolytic) cell suspension results from the serial connection of the suspension impedance $\underline{Z}_S$ and the sum electrode impedance $\underline{Z}_E$ of the two inserted electrodes. As well known, $\underline{Z}_E$ is due to phenomena of electrode polarization which will be discussed later. In order to determine both $\underline{Z}_S$ and $\underline{Z}_E$ we apply two "raster electrodes". According to Fig.2a, each of them exhibits two segments of effective sum area $A$. For the separation of sum impedance, two measurements are made which yield:

$$\underline{Z} = \underline{Z}_S + \underline{Z}_E , \qquad \underline{Z}^* = (1 + a) \cdot \underline{Z}_S + (2 + \underline{\beta}) \cdot \underline{Z}_E . \qquad (1)$$

$\underline{Z}$ is derived from galvanically connected segments, i.e. with whole electrode area $A$ (compare the vector diagram in Fig.1). $\underline{Z}^*$ results from disconnected raster halves, the effective electrode area being close to $A/2$. Here, the correction factor $a$ considers the narrowing of current flow lines in vicinity of the electrodes resulting from approximate electrical ineffectiveness of disconnected raster halves (Fig.2c). $a$ is a characteristic of the individual electrode system. Prior to the electrode's utilization for a given suspension and a given frequency $f$, $a$ is determined by a measurement on physiological saline solution with a sufficiently high frequency ("HF"; e.g., $f = 100$ kHz). In this case, the influence of $\underline{Z}_E$ is negligible with respect to correction factors, and also the imaginary component of $\underline{Z}_S$ can be neglected ($e$ being as small as 80), i.e. we measure $\underline{Z}(HF) = Z(HF) = Z_S(HF)$ and $\underline{Z}^*(HF) = Z^*(HF) = (1 + a) \cdot Z_S(HF)$ which yields

$$a = Z^*(HF) / Z(HF) - 1 \qquad (2)$$

The complex factor $\underline{\beta}$ takes into account that the reduced electrode area will differ slightly from $A/2$ due to manufacturing imperfections. It also considers that disconnection of a raster half is not

equivalent with total electrical ineffectiveness. Rather, the disconnected raster half (of high conductivity) will take over a bridging function (Fig.2d). Due to its own electrode impedance it will affect the over-all electrode impedance $(2 + \underline{\text{ß}}) \cdot \underline{Z}_E$ in a most complicated way which is considered empirically by $\underline{\text{ß}}$. As shown in the following, the permittivity of microorganism cultures is considerably smaller than that of blood, the dielectric measurement thus being more sensitive with respect to capacitive or inductive side effects. This means that both the modulus and the phase angle of $\underline{\text{ß}}$ have to be determined precisely by means of a measurement on saline solution performed at the scheduled - usually rather low - operating frequency $f$ of the measuring system. Taking advantage from the fact that $Z_S$ for saline solution can be assumed to be independent from frequency, the two results of measurement $\underline{Z}(f) = Z_S (HF) + \underline{Z}_E(f)$ and $\underline{Z}^*(f) = (1 + a) \cdot Z_S (HF) + (2 + \underline{\text{ß}}) \cdot \underline{Z}_E(f)$ yield

$$\underline{\text{ß}} = \left\{ \underline{Z}^*(f) - Z^*(HF) \right\} / \left\{ \underline{Z}(f) - Z(HF) \right\} - 2 \tag{3}$$

Of course, due to complicated physical mechanisms, $a$ and $\underline{\text{ß}}$ are not independent from each other. But since both represent correction factors, insufficiencies of the above procedures are acceptable.

The above formulae yield the complex impedance $\underline{Z}_S$ of the suspension and the impedance $\underline{Z}_E$ of the electrodes as

$$\underline{Z}_S = \left\{ (2 + \underline{\text{ß}}) \cdot \underline{Z} - \underline{Z}^* \right\} / (1 - a + \underline{\text{ß}})$$
$$\underline{Z}_E = \left\{ \underline{Z}^* - (1 + a) \cdot \underline{Z} \right\} / (1 - a + \underline{\text{ß}}) \tag{4}$$

For measurements on blood, raster electrodes have been prepared by conventional print technique, for chemical inertness the copper layers being gold and rhodium plated. For microorganism studies which involve long procedure times in order to study growth processes, solid gold or - alternatively - gold paste layers print on a ceramic base proved to be necessary. As a much cheaper and most effective alternative, layers of stainless steel were used showing the advantage of a fairly stable electrode operation point (see below). Fig.2b shows an example for the arrangement of a set of raster electrodes in a culture tube. Aiming for approximately homogeneous field conditions, the two electrodes are fixed at two inner walls of a square polycarbonate cylinder which is mounted on the screw cap. In order to avoid demands of exact filling height, two additional polycarbonate sheets (P) are arranged in the tube, restricting the current lines $S$ to the height of the raster electrodes. This proved to be advantageous also with respect to unavoidable changes of liquid level due to on-line sample taking for plate counts or other analytical methods. With these conditions the measured impedance $\underline{Z}_S$ yields the conductivity and the permittivity in sufficiently good approximation from

$$s + j\, 2\,\pi \cdot f \cdot e_O \cdot e = D / (A' \cdot \underline{Z}_S) \tag{5}$$

Here, $D$ is the distance of raster electrodes and $A'$ is the gross area of the two raster regions of an electrode.

**Figure 3.** Block diagram of apparatus.
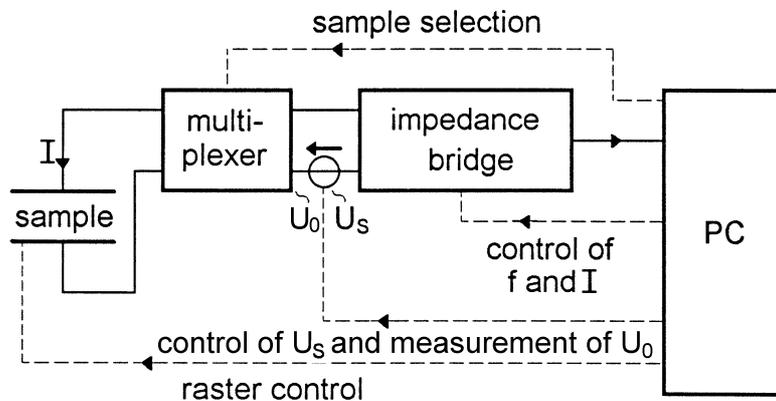


With respect to long procedure times, a system of six - thermally stabilized - test tubes was controlled by a multiplex equipment in connection with a computer allowing for automatic measurements including connections of raster halves by means of reed switches (block diagram in

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Fig. 3). The computer was also used for automatic variations of the frequency $f$ over time, the measuring current $I$ and the optionally superimposed DC voltage $U_S$ allowing for shifts of the electrode operation point.

## 3 BIOLOGICAL MATERIAL

Analyses were focused on suspensions of bacteria (Escherichia coli; mean diameter $d$ close to 1.6 µm; see paragraph 4) and yeast cells (Candida crusei, Saccharomyces cerevisiae; $d \approx 3$ µm). Some experiments were also performed on algae (Chlorella fusca; $d \approx 10$µm with strong variations). For investigations of growth processes, approximate homogeneity can be assumed to be given for bacteria due to flaggelation and for algae due to aeration. Recordings of changes of $s$ or $\underline{Z}_E$ during growth of microorganisms were made for bacteria at 37°C and yeasts at 30°C in specific growth media. Cultures of algae were mounted on a light panel and ventilated by air.

For investigations of influences of the cell concentration $n$, cells were cultivated in growth medium for 24 h. After this incubation period, bacteria cells have grown up to concentrations close to $10^9$ cells/ml. They were washed in physiological saline solution, and then the densitiy was increased by centrifugation procedure up to about $3 \cdot 10^{11}$ cells/ml for bacteria and up to $3 \cdot 10^{10}$ for yeast cells. According to the respective demands of investigations, a stepwise re-suspension allowed adjustments of any desired cell concentration $n$ which was determined by means of the conventional plate count method, centrifugation or by microscopic counting. Measurements on re-suspensions were performed under homogeneous conditions.

## 4 ANALYSES OF HIGHLY CONCENTRATED MICROORGANISMS

In first series of measurements, the cell concentration was varied step by step facilitating re-suspension of highly concentrated cells. Then the electric measurement was performed without delay to prevent effects of metabolism. Fig.4 shows typical results for the conductivity $s$ and the permittivity $e$ as a function of $n$, determined by microscopic counting. For both bacteria and yeast cells, with increasing $n$, $s$ proved to be almost constant for $n < 10^9$/ml (Fig. 4b). With further increase of $n$, $s$ showed decreases which were more pronounced for yeast cells. According to Fig. 4a, $e$ showed contrary behavior. From extrapolation, $e$-values of yeast cells approach the order of 1000 for $n$ close to $3 \cdot 10^{10}$ cells/ml. For bacteria cells, the results of $e$ were of the order 100 for low $n$ - as to be expected for the aqueous liquid. Strong scatter has to be expected here a priori due to both strong effects of unavoidable phase errors and insufficiencies of the raster electrode technique discussed in paragraph 2. For $n$ exceeding $10^{10}$ cells/ml, $e$ increased strongly, finally also reaching the order of 1000.

Qualitatively, the above results are in full correspondence with low-frequency results obtained from suspensions of other cell types, e.g. erythrocytes [10], tumor cells [12], yeast or bacteria cells [15,20,21]; i.e. increasing $n$ yields decreasing $s$ due to the low conductivity of membrane structures, and charging of the latter yields the respective increases of $e$. Quantitative interpretations of results can be based on classic theories of ß-dispersion [12]. However, attempts of precise modeling fail a priori due to the indefinable system: non-spherical cell shapes, scatter of cell sizes, complex molecular structure of bacteria cell membranes and walls, etc. Neglecting both the conductivity of the latter - which seems to be justified using a good conducting medium (according to [12]) - and the permittivity of the intracellular region, the conductivity and the permittivity of the suspension can be expressed in rough approximation as

$$s = s_L \cdot (1 - n_V) / (1 + n_V / 2) \quad , \qquad e = e_L + 9 \cdot e_M \cdot d \cdot n_V / \left\{ 8 \cdot d_M \ ( 1 + n_V ) \right\} \qquad (6)$$

Here, $s_L$ is the extracellular liquid´s conductivity and $e_L$ the permittivity of both, the extracellular and the intracellular liquid. $e_M$ is the membrane's permittivity, $d_M$ the membrane's thickness and $n_V$ the volume fraction of the suspended cells.

Assuming a mean diameter $d = 3$ µm for yeast cells, there results $n \approx 7 \cdot 10^8$ cells/ml for $n_V = 1\%$, i.e. the concentration at which significant decreases of $s$ and increases of $e$ should start. For bacteria cells the respective minimum concentration is shifted to $5 \cdot 10^9$ cells/ml (assuming a volume of a 1 µm x 3 µm rod like shape of $2 \cdot 10^{-18}$ m$^3$, equivalent to a spherical diameter $d = 1.6$ µm). The results of Fig. 4 indicate that significant changes start at even lower concentrations. However, even much stronger effects of low bacteria concentrations on $s$ have been reported in [22], where significant changes are indicated for $n$ close to $10^3$ cells/ml, the cells being suspended in distilled water. Still, the physical mechanism of these phenomena has not been discussed in [22] thus being questionable.

For yeast cells, maximum concentration was close to $n_V = 40\%$. According to Fig.4 this yielded about 30% decrease of $s$ in comparison to $s_L$. This decrease is less than expected by (6) which might be due to the above mentioned fact that the latter neglects membrane conductivity. The corresponding measured value $e \approx 900$ would agree with the result of (6) if we assume a membrane thickness $d_M = 8$ nm in connection with $e_M = 8$ - assumptions which are quite reasonable. The results also agree with [23] were values of $e$ are reported in the order of 480 and 1080 for $n_V$-values of 10% and 40%, respectively. On the other hand, $e \approx 1500$ has been reported in [20] for $n_V$ not exceeding 20% and in [21] for $n_V$-values varying between 40% and 55%.

For bacteria, maximum $n_V$ was close to 60% corresponding to 35% decrease of $s$ which again is less than theoretically expected. The corresponding maximum values of $e$ were close to 800. Assuming membrane characteristics as in the case of yeast cells, (6) would yield 750 due to the small (mean) diameter of bacteria. According to [24], E.coli suspensions yielded values of $e$ in the order of 1000 for cell densities of $5 \cdot 10^{11}$ cells/ml and a frequency of 50 kHz. Contrary, [13] reports values of $e$ (at 50 kHz) close to 2000 for Micrococcus lysodeikticus ($n_V = 30\%$). Such differences are not surprising if we consider both experimental difficulties and the very strong scatter of yeast cell sizes. In addition, problems of electrode polarization arise in the low frequency range, a problem which is considered by different authors in a quite different way, explaining strong scatters of calculated $e$ -values.
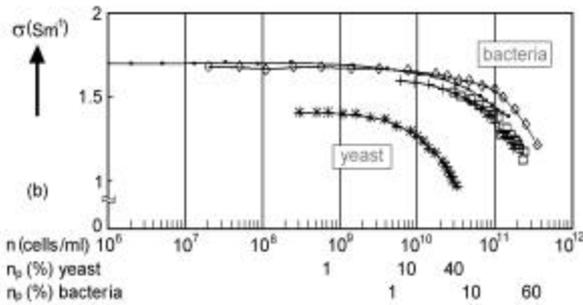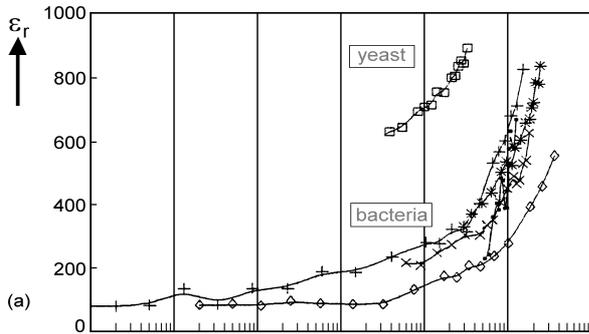


**Figure 4.** Dielectric properties of suspensions of yeast and bacteria cells, respectively, as a function of the cell concentration. (a) Permittivity. (b) Conductivity.

## 5    MONITORING OF GROWTH PROCESSES

A second series of measurements performed during the present study was based on very small initial concentrations $n_0$, the electric measurement being extended over several hours, thus detecting effects of growth processes including metabolism. In these investigations, strong emphasize was also put on characteristics of the electrode system which has been shown to be affected by metabolic activities as well [16, 25]. The growth process is represented by a continuous increase of the cell concentration and additionally by changes of the ionic content of the nutrient liquid due to metabolic activities which cause both changes of ion concentrations and modifications of ionized molecules - and thus also changes of their effective mobility as well as of their polar characteristics.

Attempts to investigate respective changes of $e$ failed due to the fact that physiological growth processes involve small values of $e$, variations which are below the resolution of the here applied method of measurement. However, we can assume that metabolic activity will not affect the first term of (6) in a considerable way due to the predominant role of the polar water molecules (yielding the order of 80). The second term will increase somewhat proportionally with $n_V$, the denominator being constant for $n_V << 1$. This means that the results of Fig.4a will be valid in principle.

On the other hand, with respect to conductivity, the results of Fig.4b are invalid since metabolism predominantly affects the factor $s_L$ of (6). For $f = 500$ Hz, Fig.5 shows typical relative changes of $s$ determined for bacteria, yeast, and algae. As also demonstrated by many other studies, there result strongly differing conductivity changes which can specifically be related to the respective metabolic mechanisms of the considered type of microorganism [26, 27]. For example, an increase of $s$ reflects increasing ionic concentrations and/or increasing ionic mobility in correspondence to the involved type
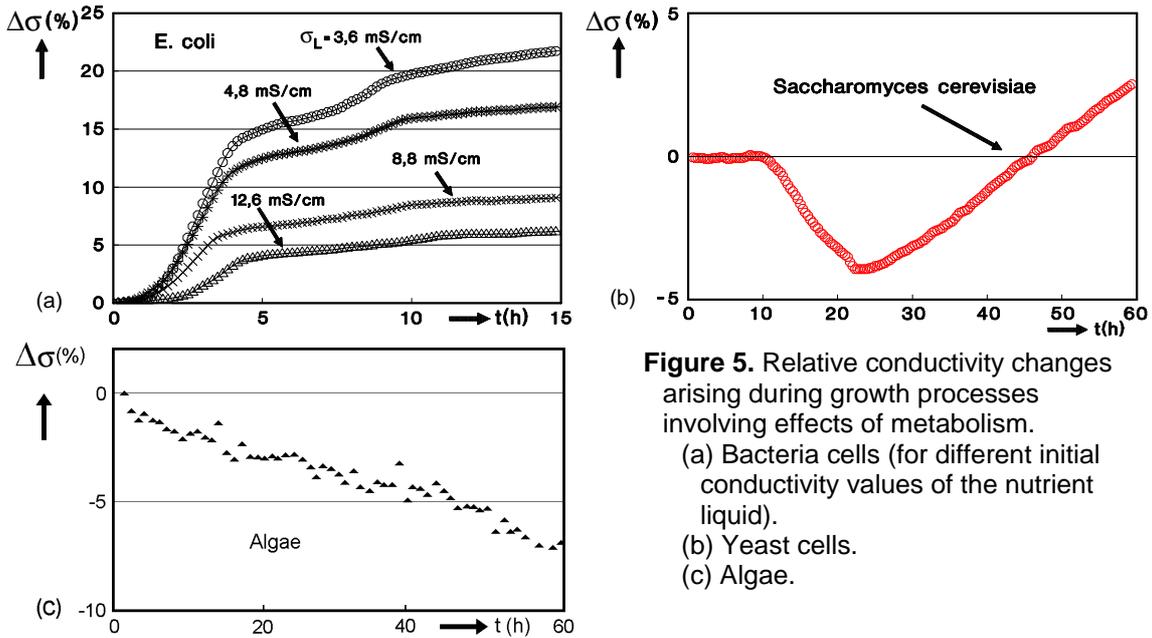
**Figure 5.** Relative conductivity changes arising during growth processes involving effects of metabolism.
(a) Bacteria cells (for different initial conductivity values of the nutrient liquid).
(b) Yeast cells.
(c) Algae.

of metabolism, discontinuities of $s(t)$ indicate a change of type of metabolism. As well known, this behavior is utilized for routine checks of contamination, the starting time of conductivity changes representing an inverse measure for the degree of contamination, i.e. of $n_0$ [28].

As already mentioned above, metabolism also affects the electrode impedance in a significant way. By means of (4), the electrode impedance $\underline{Z}_E$ has been determined in addition to $\underline{Z}_S$ for all measurements in the framework of this study. According to theory and as demonstrated by Fig.6a, the modulus of $\underline{Z}_E$ proved to decrease strongly with increasing frequency $f$ and slightly with increasing current density $S$ - the respective decrease however being most pronounced for low $f$ and $S$-intensities below the critical value (close to 1 mA/cm$^2$). On the other hand, the phase angle $j_E$ of $\underline{Z}_E$ was of the order of -70$^O$ in all cases (provided $S < 1$ mA/cm$^2$) which means that it does not represent substantial information. Thus attention was focused on the modulus $Z_E$ which proved to exhibit strong variations also with respect to the electrode material. Optimum results were attained for well aged stainless steel electrodes.
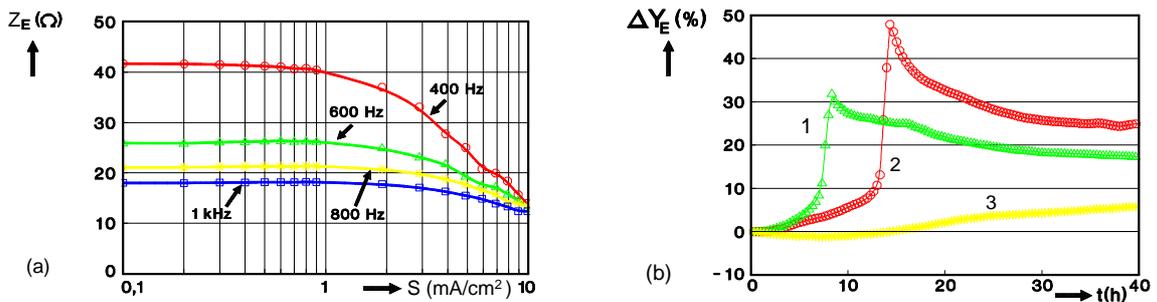


**Figure 6.** Typical behavior of electrode impedance. (a) Modulus of electrode impedance as a function of current density and frequency as being typical for standard E. coli nutrient liquid. (b) Spike-like responses of electrode admittance which offers a most effective detection of yeast cells (curve 1 for high initial concentration, 2 for lower concentration and 3 for sterile medium).

As already reported earlier [7,25], we found that a registration of time changes $Z_E(t)$ represents the most effective tool for microorganism detection especially in the case of high a priori conductivity of the nutrient liquid. To facilitate comparisons with microbiological literature, it proved to be advantageous to display variations of the electrode admittance $Y_E = 1 / Z_E$. Fig.6b shows a typical result for yeast cells. As an additional advantage of this method, the responses prove to be considerably higher than in the case of conductivity (up to about 50% instead of about 20%).

As well known, the modulus $Y_E$ may be distinctly affected by the so-called steady state electrode contact voltage $U_0$ (e.g. [29]). For a clarification of this influence, we measured $U_0$ during microorganism growth against a reference electrode (Hg/HgSO$_4$). Further, we varied the value of $U_0$ by a

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superimposed voltage $U_S$ (see block diagram in Fig. 3). Fig. 7a shows time courses of $U_0$ (related to the normal hydrogen electrode) measured on stainless steel electrodes during growth of three different bacteria species. The arrows in Fig. 7a show the start of exponential growth. While for Pseudomonas aeruginosa a distinct drop of $U_0$ from 130 mV to about 30 mV was observed, only small rises of $U_0$ of about 30 mV occurred for the species E.coli and Staph. aureus. Fig. 7b shows time courses of $U_0$ during growth of E. coli registered by different types of metal electrodes. For example, the most pronounced change of $U_0$ from 200 mV to -300 mV was measured for platinum electrodes.
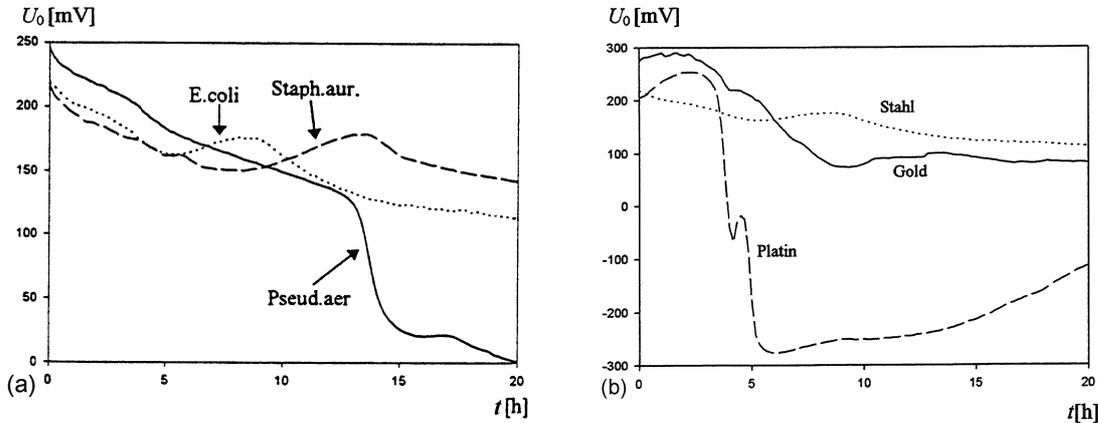
**Figure 7.** Time courses of the steady state electrode contact voltage $U_0$ during growth of different bacteria. (a) $U_0$ measured with stainless steel electrodes and different bacteria species. (b) $U_0$ during growth of E. coli, measured with different electrode metals.

For chloride solutions and different culture media, the influence of a superimposed voltage $U_s$ is demonstrated by Fig.8a. As a practical consequence, variations of the initial voltage $U_0$ may have different effects on the time response $Y_E(t)$ during a growth process. For example, Fig.8b shows strong effects of $U_S$ on results for E. coli cultures.
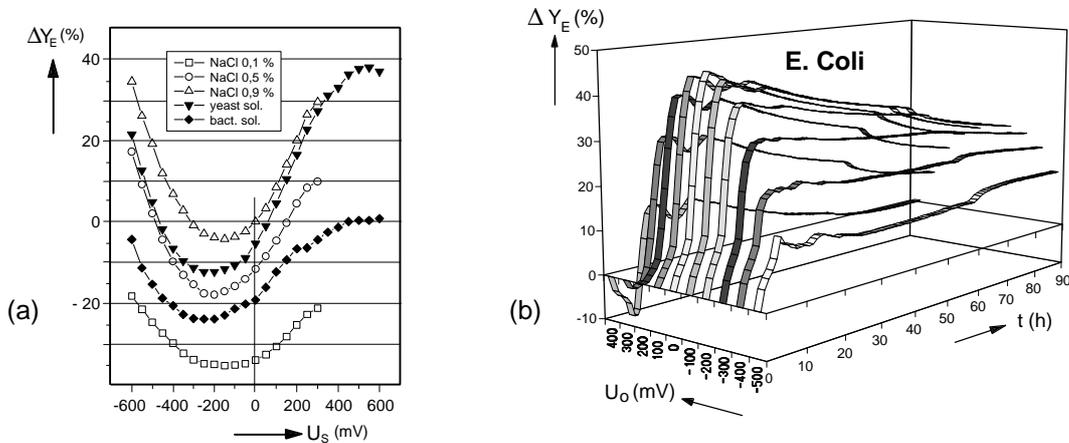
**Figure 8.** Typical effects of a superimposed DC voltage yielding shifts of the electrode´s steady state voltage. (a) Percentage change of the electrode admittance for the case of a 0,9% saline solution and zero shift. Additional results are given for 0,1% and 0,5% saline solutions as well as for nutrient solutions for yeast cells and bacteria cells. (b) Effects of a superimposed DC voltage on the admittance´s time response during the growth process of E. coli.

# 6 CONCENTRATION OF MICROORGANISMS BY DIELECTROPHORESIS

As shown in chapter 4, the electric quantities $e$ and $s$ can be used for quantifications of highly concentrated microorganisms in fields of industrial fermentation processes. On the other hand, e.g. in food products or drinking water, very low numbers of microorganisms arise. Here, the detection can only be attained by continuous measurement during defined incubation as described in chapter 5.

In the present work, we developed a new automatic method for accelerated detection. It is based on a regional concentration of microorganisms within the sample chamber by means of dielectrophoresis. A plane, star-like electrode system is used in connection with a high frequency electric field (500 Hz -

1 MHz). Fig. 9 shows an example for the concentration process monitored by light microscopy in transmission mode. Fig. 9a shows the start of the process of concentration of E. coli bacteria. Bacteria of the peripheral region are guided towards the central ends of the electrodes in direction of higher field strength. In Fig. 9b and Fig. 9c, an advanced concentration is visualized after 1 min and after 5 min, respectively. For the detection of the initial bacteria concentration it is planned to arrange an additional spot-like electrode in the center of the system.
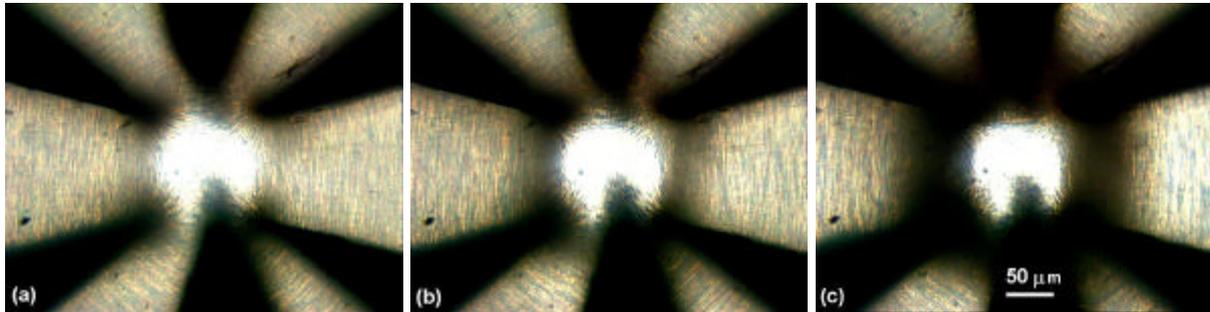


**Figure 9.** Computer images of a star-like electrode arrangement for the dielectrophoretic concentration of bacteria striving for accelerated detection. (a) Start of the concentration. (b) Aggregation of bacteria in the center of the electrode system after about 1 min. (c) After 5 min.


## 7    CONCLUSIONS
The results of the present study yield the following main conclusions:
(i) A raster electrode method which has been initially developed for analyses of blood proves to be an effective tool also for microorganisms.
(ii) The method allows for an instantaneous detection of bacteria and yeast provided that the initial concentration $n$ exceeds the rough order of $10^8$ cells/ml.
(iii) In cases of low $n$, the detection can be performed during incubation through a measurement of time changes of the culture medium´s conductivity.
(iv) In cases  of high a priori conductivity of the nutrient liquid, most effective detections prove to be offered by a measurement of time changes of the electrode impedance.
(v) The registration of the electrode contact voltage $U_0$ offers an additional information for specific detections of individual species.
(vi)  Well defined changes of $U_0$ by a superimposed, specifically adapted voltage $U_S$  offer a tool for the control of the electrode´s effective sensitivity.
(vii) A pre-concentration of microorganisms by means of dielectrophoresis and a subsequent measurement of the electrode impedance and/or contact voltage promises to allow an accelerated detection of microorganisms of very low initial concentration $n$.


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