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Low frequency electric polarizability and zeta-potential of *Escherichia coli* HB101 (K-12) cells during inactivation with ethanol

Research Article

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Abstract: The electric properties of bacteria determine their non-specific interactions with the environment, in particular their pathogenic activity. The electric polarizability of *Escherichia coli* HB101 (K-12 strain) was studied while inactivation with ethanol (20-40 vol.%). The current investigation might be regarded as a continuation of previous research on the polarizability of *E. coli* at lower ethanol concentration (≤ 20 vol.%) and higher frequencies (≥ 20 kHz).

The bacteria polarizability at low frequencies ($<10^4$ Hz) shows anomalies (unexpected increase in the polarizability at certain ethanol concentrations), while the parameter decreases with an increase in the ethanol concentration at higher frequencies. We investigated for the possible reasons causing the anomalies – in our case reduced to the medium dielectric permittivity, the average cell length and the surface electric charge density distribution, related to bacterial lipopolysaccharides. We suggest a hypothesis for the molecular mechanism of changing the surface charge of *E. coli*, carried by lipopolysaccharides, induced by the non-ionic ethanol.

Keywords: *Electric light scattering (ELS) • Microelectrophoresis • Dynamic light scattering (DLS) • Escherichia coli K-12 • Lipopolysaccharides*

1. Introduction

The electric properties of bacteria determine the non-specific interactions with the environment, in particular their pathogenic activity (*e.g.* attachment to the host's cells). Therefore, their inactivation could be also related to changes in the electric properties.

A major component of the outer surface layer of the membrane of *Escherichia coli* contains lipopolysaccharides (LPS). The LPS molecule of the non-pathogenic strains, such as K-12, consists of a hydrophobic lipid A (endotoxin) and hydrophilic inner and outer core. The pathogenic strains also include repeating units of O-antigen found in the architecture of LPS [1].

According to J. Klena *et al.* [2], the core region of LPS contains a ligand that enables the binding the bacterium to special surface receptors of dendritic cells The O-antigen the role of shielding component only. That is why the non-pathogenic K-12 strain could be considered as a model of pathogen, *i.e.*, a harmless object for investigation of the properties of the pathogenic strains.

The *E. coli* negative electric charge and its value are determined by the core of LPS. The core of the LPS is the location of the functional groups [3]. That region is of great importance in determining the bacteria interactions with the environment. By removing the core phosphate groups, for instance, results in avoiding an immune response to the pathogen and resistance to antibiotics [4].

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According to Latrache *et al*. and Mozes *et al*. [5,6] the phosphate groups of LPS play a central role in the surface charge development while the of carboxyl groups have a minor influence. The zeta potential is also partially deter\ined by the negatively charged phospholipids of *E.coli* [7,8].

Studies of the electric properties of bacteria during their inactivation are reported in the literature [9-18].

Morris and Jennings [10-12] carried out detailed investigations on the effect of various additives (antibiotics, alcohols and others) on the electric polarizability of bacteria (including different strains of *E. coli*). They observed a strong decrease in the polarizability for all additives studied. According to Miroshnikov *et al*. [9] all the inactivation factors impact the barrier functions of the cell membrane.

In our previous study [19] we investigated the influence of ethanol with a range of concentrations between 0-20 vol.% on the high frequency (20 kHz – 20 MHz) electric polarizability of *E. coli* K-12. At that high frequency range, Maxwell-Wagner polarizability (MWP) occurs [20,21]. This effect characterizes the electric properties of the membrane itself and the distribution of the inner (cytoplasmic) electric charge [9]. We have found that high frequency polarizability decreases linearly with an increase in the ethanol concentration [19]. This result was attributed to the ethanol causing an increase in the membrane permeability which followed a decrease in the cytoplasm electric conductivity. The decrease in the medium permittivity in presence of ethanol was suggested as an additional reason for the polarizability change.

Ethanol concentrations between the range of 0-20 vol.% does not inactivate the bacteria, while the range of 20-40 vol.% decreases significantly the viability of *E. coli* [9]. This makes the higher concentration range interesting for studying the changes in the electric properties of bacteria *E. coli* during their inactivation. The latter is a main topic of discussion in the present paper.

Additionally, Miroshnikov *et al.* [9] observed "variability" in the low frequency polarizability when adding an inactivating agent (such as ethanol). At low frequencies (≤10^5 Hz), charge-dependent polarizability (ChDP) occurs [21]. It is related to the electric properties of the outer surface and its double electric layer (DEL). The "variability", mentioned above, was meant as an increase in the polarizability value at some values of ethanol concentration, although it decreases in general with the increase in the ethanol concentration. The authors did not investigate this phenomena and do not suggest an explanation for these deviations from the behavior of the parameter compared to that at higher frequencies. This unanswered question will also be investigated in the present article; the reason for this "variability" of the electric polarizability at low frequencies (10 Hz – 20 kHz).

2. Experimental procedure

2.1. Cultivation of *E. coli* HB101 and preparation for investigation

The bacterial strain *E. coli* HB101 (from the laboratory collection of The Stephan Angeloff Institute of Microbiology, Sofia) was cultivated for 24 h in Luria Bertani (LB) broth (10 g tryptone, 5 g yeast extract, 10 g NaCl, pH adjusted to 7.0 with 1 M NaOH) at 37°C. The bacterial cells harvested by centrifugation (5000 g/10 min) were washed twice in and re-suspended in tri-distilled water to obtain 10^7 CFU mL^{-1}. The number of cells were determined by the McFarland standard and by the classical plaiting method using LB agar (1.5% w/v).

The *E. coli* HB101 culture was stored at −20°C in LB broth supplemented with glycerol (20% v/v). It was used as the initial inoculum (1% v/v) for the preparation of *E. coli* culture in the stationary phase, which was synchronized before the assay by doubly pre-cultivation in LB broth for 18 h at 37°C, under intensive aeration.

Some of the experiments were performed by fixing *E. coli* HB101 with formaldehyde. For those experiments, the following procedure was applied, equal aliquots of 100 mL of culture E. coli HB101 (in the stationary phase) were doubly washed with deionized, tri-distilled water. The cells were pelleted by centrifugation (8000×g) and were suspended in a 3% aqueous solution of formaldehyde. Before the experiments, the bacteria was separated from formaldehyde using a milliporous filter (pore size 0.45 μm) and then suspended in distilled water.

2.2. Electric Light Scattering (ELS)

Applying an electric field to a bacterial suspension induces a change in its optical properties and results in a re-orientation of the bacteria. The electric light scattering (ELS) effect, also called electro-optical effect (α) is defined by the relative change in the light scattering intensity (I) in presence (I_E) and in absence (I_0) of electric field E in the suspension (Fig. 1) [20]:

$$\alpha = (I_E - I_0) / I_0 = \Delta I / I_0 \qquad (1)$$

In our studies, the light scattered at 90° was recorded.

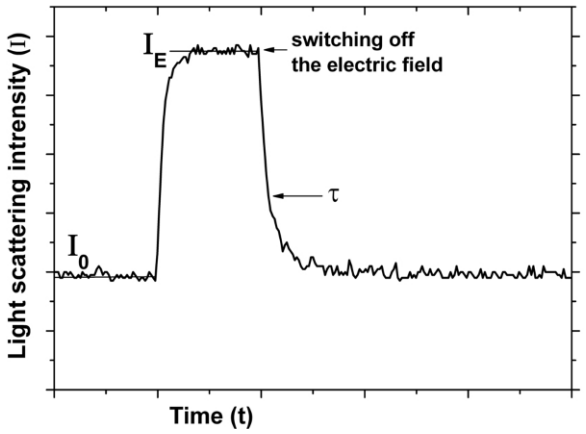

Figure 1. Electro-optical pulse: Light scattering intensity (*I*) as a function of the time (*t*).

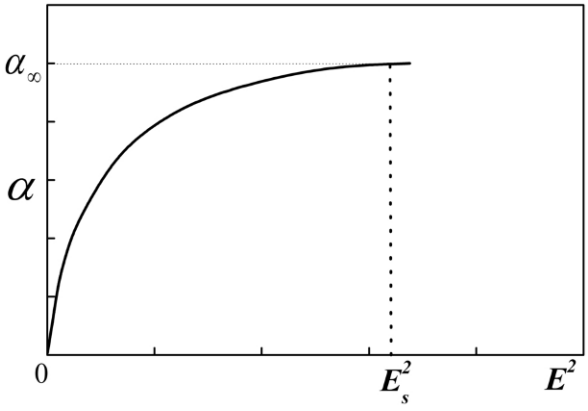

Figure 2. The electro-optical effect *α* as a function of the electric field intensity *E*; E_S - the value of the electric field intensity at the point of saturation of the effect.

The relaxation time of the disorientation, τ, is determined by the time after switching off the electric field, for which the electro-optical effect decreases *e*-times (*e* = 2.72). It is related to the rotational diffusion coefficient D_r of the bacteria:

$$\tau = 1/6D_r \tag{2}$$

In the case of a rotational ellipsoid (to which the bacterial cell is approximated) with semi-axis *a*, *b* = *c* and axial ratio $p = a/b$, the value of D_r is given by the Perrin's formula [22]:

$$D_r = \frac{kTp^2}{4\eta V\left(p^4-1\right)}\left[-1+\frac{2p^2-1}{2p\sqrt{p^2-1}}\ln\frac{p+\sqrt{p^2-1}}{p-\sqrt{p^2-1}}\right] \tag{3}$$

where $V = \pi ab^2/6$ is the volume of the bacterium, η is the viscosity of the medium.

The electric polarizability γ of bacteria is calculated from the electro-optical effect α by the dependence (Fig. 2): $V = \pi ab^2/6$

$$\gamma = \frac{\alpha}{\alpha_\infty E^2} \tag{4}$$

where α_∞ is the value of α at high values of the electric field (saturation of the effect), where the effect does not depend anymore on *E* [20].

The electric polarizability, γ, is a function on the frequency, ν, of the electric field. Depending on the frequency of relaxation, different dispersions, appearance and mechanisms of polarizability were observed. Additionally, at $\nu \geq 10^9$ Hz, volume polarizability is observed, butat lower frequencies interfacial polarizability appears. There are two main types of interfacial polarizability: Maxwell-Wagner polarizability (MWP) and Charge-dependent polarizability (ChDP). ChDP occurs at lower frequencies (≤10^5 Hz), where the electric properties are related to the bacterium surface and its DEL. MWP relaxes at higher frequencies (10^6-10^7 Hz) and characterizes the membrane itself and the distribution of the inner (cytoplasmic) electric charge.

$$\gamma(ChD) = f(1/\kappa, L, q) \tag{5}$$

$$\gamma(MW) = f(\varepsilon, \varepsilon_m, K, K_m, V) \tag{6}$$

where 1/k is the thickness of the Double electric layer (DEL), L and q are the length and the electrokinetic electric charge density of the bacteria, assuming its uniform distribution over bacterial surface, ε and ε_m are the values of the dielectric constant of the cell and the medium, K and K_m are the values of the electric conductivity of the cell and the medium, *V* is the volume of bacteria [21]. Additionally, 1/k depends on e_m and the volume electrolyte concentration C_∞ in the medium [23]:

$$1/\kappa = \sqrt{\frac{\varepsilon_m RT}{8\pi z^2 F^2 C_\infty}} \tag{7}$$

T is the absolute temperature, z is the ions' valence; and R and F are the gas constant and the Faraday constant respectively.

2.3. Microelectrophoresis measurements

The electrophoretic mobility (EPM) was measured using a cytopherometer (OPTON, Feintechnik GmbH, Wien, Austria) with a rectangular cell and platinum electrodes. The electrophoretic migration of 15 - 25 particles was timed for both forward and backward (reversed field) runs over a known distance (80 μm) at a constant electric current of 4 μA, voltage of 540 V (fixed *E. coli* cells), as well as of 6 – 8 μA, 530 V (live *E. coli* cells).

The *E. coli* cells were suspended in deionized water and in water-ethanol medium at a constant concentration of 1×10^7 cells mL^{-1}. The bacteria were observed under a light microscope, connected to a Sony video camera, providing 800-times magnification. The observation light (with intensity of 920 μmol quanta m^{-2} s^{-1}) was filtered through a blue-green interference filter. The average bacterial size of about 3 μm was registered. The images were recorded on a Sony video recorder RDR-GX700/S and the results were expressed as a mean of the EPM per 10^{-7} m^2 V^{-1} s^{-1} ± standard error for each probe. The standard errors in the measured electrophoretic mobility, *u,* were between 2-5%. The electric conductivity of the suspension medium was measured using a Cyber Scan PC510 (Eutech Instruments, USA, Singapore) pH/Conductivity meter. Its value was 2.14 μS cm^{-1}. All experiments were carried out at 25°C.

The zeta (electrokinetic) potential, ζ, was calculated from the electrophoretic mobility *u* using Helmholtz-Smoluchowski equation [24]:

$$\zeta = \frac{4\pi.\eta\times u}{\varepsilon_m \times \varepsilon_0} \qquad (8)$$

where ζ is in units of mV, ε_m is the dielectric constant of the aqueous phase, ε_0 is the permittivity of the free space ($\varepsilon_0 = 8.8542\times10^{-12}$ Fm^{-1}), and η is the viscosity of the aqueous phase.

2.4. Particle size analyzer

The size of the *E.coli* cells were measured with a Zetatrac™ instrument. This device can measure particle size ranging from 0.8 nm to 6.5 μm with polarity. The Zetatrac™ uses the dynamic light scattering method. The velocity distribution of a sample of particles suspended in a medium is a known function of the particle size [25]. Light from the laser diode is coupled to the sample through an optical power splitter assembly. Light scattered from each particle is Doppler-shifted by particle motion (Brownian motion). The Doppler-shifted scattered light is mixed with coherent, uni-shifted light; and the optical system sends these mixed signals to a silicon photo-detector. The detector output signal is then amplified, filtered, digitized, and mathematically analyzed by Microtrac ® FLEX Windows Software, using proprietary algorithms, to provide the particle size distribution. For measuring particle size distribution, the *E. coli* cells were properly dispersed in the solvent using Vortex. The ionic strength of the deionized water used in these experiments is reported as 1×10^{-7} M [25].

3. Results and discussion

3.1. Dependence $\gamma = f(C_{ethanol})$ for live *E. coli* HB101 at various concentrations of ethanol (20-40 vol. %) and at low frequencies (10 Hz – 20 kHz)

The frequency dependence of the electric polarizability (γ) of *E. coli* HB101 (K-12) was previously studied [26,27]. These studies have included low frequency plateaus (in the range 10-100 Hz) and a high frequency maximum (at about 0.6-1 MHz). The influence of ethanol concentrations between 0-20 vol.% on frequency maximums, reported in [19], showed a linear decrease in γ with increasing the ethanol concentration. In the low frequency range, it is appropriate to take the value of γ at the plateau (which does not depend on the frequency for certain range) as a function of the concentration of ethanol.

Fig. 3 shows the frequency dependencies of γ of live *E. coli* HB101 cells at different concentration ethanol in the range 20-40 vol. %. The curves at 0 and 10 vol.% ethanol are shown for comparison. The polarizability is calculated from the electro-optical effect by Eq. 4.

As expected, the changes in γ at low frequencies (10 Hz – 20 kHz) are not as significant as those at higher ones (20 kHz – 20 MHz) [9,19].

The plateau in Fig. 3 shows a frequency of 100 Hz at ethanol concentration between 0-30 vol. % and a frequency of 20 Hz at 35 and 40 vol.%. When the polarizability plateau is referenced within this study, it is meant that these frequencies correspond to these values of ethanol concentration.

Previous studies have interpreted the plateau with the occurrence of ChDP [26,27]. In Fig. 3 the plateau shifts to lower frequencies with an increase in the ethanol concentration, which has also been observed at higher frequencies and lower concentration of ethanol [19]. The fact has been attributed to a decrease in the internal MWP as a result of a decrease in the internal electric conductivity of the cells in presence of ethanol. However, at lower frequencies the shift might be also related to changes in the average length of bacteria, on which ChDP is strongly dependent [27].

Fig. 4 provides evidence that γ decreases with the increase in the concentration of ethanol between the range of 0-20 vol.%. This was also observed for higher frequencies. The reference polarizability value thatwe selected for this study was set at 20 kHz – this frequency was out of the plateau range and the highest one for the current experiment (limited by the ELS device). However, some interesting changes (anomalies) in γ of

the plateau are observed at 25-40 vol.% of ethanol. At 25 vol.% of ethanol, γ does not decrease, but maintains nearly equal to that at 20 vol.%. At 35 vol.% ethanol, γ increases compared to that of 30 vol.%. Furthermore, these deviations seem to appear especially at the lower frequencies (at the low frequency plateau), but not at 20 kHz (Fig. 4). We are going to study the anomalies (also observed by [9] and called "variability" of γ) in the present paper and search for the reason, which causes them.

3.2. Dependence of $\gamma = f(C_{ethanol})$ for fixed *E. coli* HB101 at concentration of ethanol 20-40 vol. % and at low frequencies (10 Hz – 20 kHz)

Because anomalies appear in the concentration range of inactivation of *E. coli* (20-40 vol.%), they could be due to some molecular changes related to viability loss. To check this possibility we investigated the same dependence, but using dead (fixed with formaldehyde) bacteria (Fig. 5).

Fig. 5 shows clearly that the anomalies appear in the dependence of γ on the ethanol concentration and also in the case of fixed bacteria. This dependence is compared to that of live bacteria in Fig. 6. The value of γ of fixed cells remain the same at concentrations of 25 and 30 vol.% ethanol, although in the case of live cells this happens at 20 and 25 vol.%. The anomaly of γ for the fixed *E. coli* cells at 35 vol.% ethanol is even more significant than that of the live ones. Despite the differences in the anomalies in Figs. 4 and 5, their appearance in both the live and fixed bacteria indicates that the inactivation mechanism of ethanol may be also related to molecular changes unrelated to losing viability.

3.3. Possible reasons for the anomalies of the dependence $\gamma = f(C_{ethanol})$ at 20-40 vol.% ethanol and low frequencies

As mentioned in section 2.2, charge-dependent polarizability (ChDP) occurs at low frequencies (≤10^5 Hz). According to Eq. 5, ChDP depends on the thickness 1/k of DEL, the average length, *L,* of the particles (cells) and the surface (electrokinetic) electric charge density of the bacteria, *q,* of the particles. Additionally, 1/k depends on the permittivity, ε_m, of the medium and the bulk concentration C_∞ of electrolyte in the suspension (Eq. 7): $1/k = f(\varepsilon_m, C_\infty)$. In our case C_∞ is practically constant, therefore $\gamma(ChD) = f(\varepsilon_m, L, q)$. We are going to investigate each of these dependencies in searching of the reason for the mentioned anomalies.

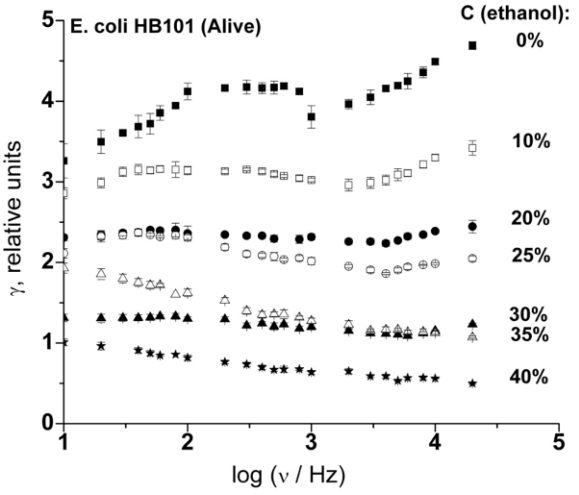

Figure 3. Frequency dependencies of the electric polarizability (γ) of *E. coli* HB101 (live) cells at different ethanol concentration in the range 0-40 vol.%.

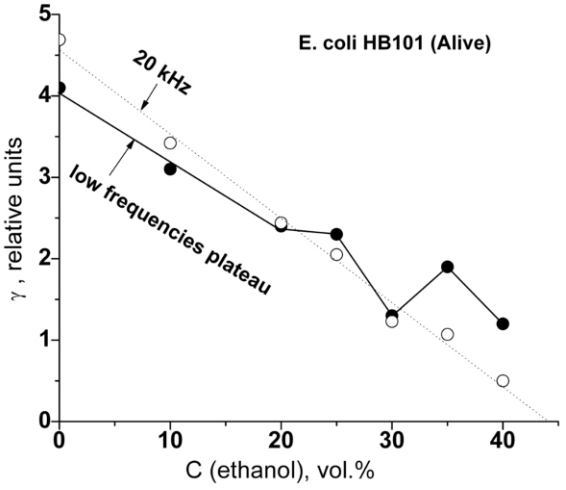

Figure 4. Dependencies of the polarizability (γ) of *E. coli* HB101 (live) cells at the plateau of the frequency dependencies (Fig. 3) on the concentration of ethanol at low and highfrequencies.

Additionally, according to literature data [28], the viscosity, η, of the water-ethanol medium increases 162% with an increase in the ethanol concentration up to 40 vol.%. Because the bacteria concentration is constant in the current case, we consider the increase in the value of η is due to the presence of cells in the water-ethanol medium, as constant and therefore unimportant to the purpose of the study.

The increase in η of water-ethanol medium does not change the stationary value of the electro-optical effect (α), from which γ is calculated, but it does change the time for orientation of the cells in electric field and that for their disorientation after switching off the field. The time, τ, of disorientation of the bacteria is used for calculating the average size of the cells (Eqs. 2 and 3).

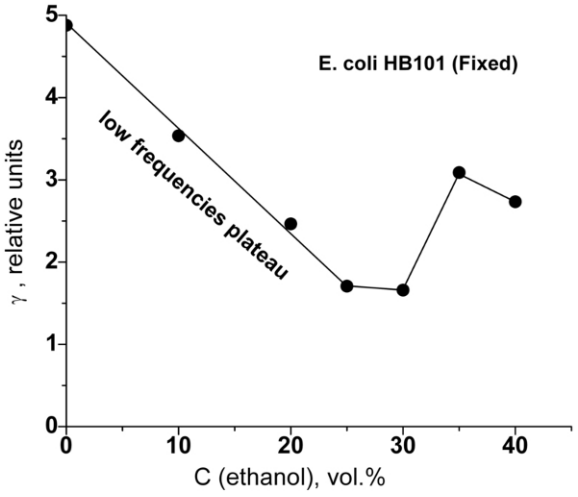

Figure 5. The dependence of the polarizability (γ) of *E. coli* HB101 (fixed) cells on the concentration of ethanol at low frequencies.

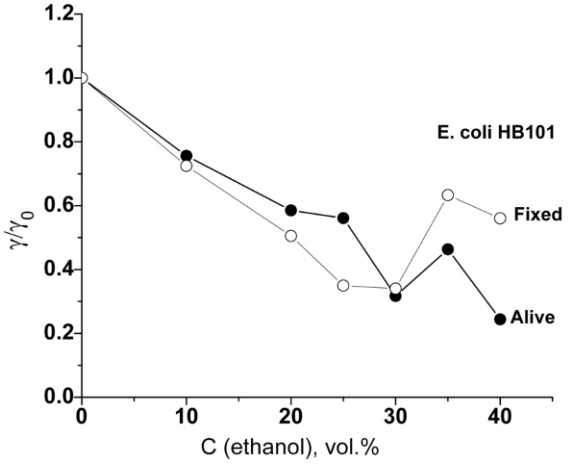

Figure 6. Dependencies of the relative electric polarizability (γ/γ_0) of *E. coli* HB101 on the concentration of ethanol at low frequencies for live and fixed bacteria; γ_0 is the polarizability γ at 0% ethanol: γ_0(live) = 4.1 rel. units, γ_0(fixed) = 4.9 rel. units.

The zeta-potential of the cells (determined by the surface electric charge density of the bacteria *q*) also depends on, η, which represents the force of resistance of the medium the particles moves through (electrophoresis). In the calculations of the length, *L,* the zeta-potential of the bacteria is taken into account and the change in the medium viscosity, η, with the change in the ethanol concentration.

3.3.1 Changes in the permittivity ε_m of the medium

It is known [29] that the permittivity ε_m of water-ethanol medium decreases by ~ 28% with the increase in the ethanol concentration up to 40 vol.%. The relative polarizability of the bacteria and relative permittivity of the medium (divided to their values at 0% ethanol) are presented as functions of the ethanol concentration in Fig. 7. The comparison of these dependencies show that a general decrease in γ may be partially caused by the decrease in ε_m. However, the linear decrease in the latter parameter cannot be the sole reason for the nonlinear anomalies in the γ-dependence.

3.3.2. Changes in the average length L of bacteria

The average length of the cellswere determined by two independent methods – ELS (from the relaxation time of the electro-optical pulse and using Eqs. 2 and 3) and DLS. As it was mentioned, the changes in η are taken into account in the calculations.

Fig. 8a shows the average bacteria length *L* (determined by ELS) as a function of the ethanol concentration for both the live and fixed *E. coli* HB101 cells. Some variations in *L* appear in presence of ethanol. However, there is evidence of a dependence on the concentration of ethanol. Obviously, there is no correlation between the *L*- and γ-dependencies at the concentration range 20-40 vol.% ethanol.

The results obtained by DLS (Fig. 8b) confirm the lack of such dependence. However, the deviations in this case are higher.

3.3.3. Changes in the surface electric charge of bacteria, represented by their zeta-potential (ζ)

The zeta-potential (ζ) of live and fixed bacteria was measured as a function of the ethanol concentration. As mentioned, the changes in η are taken into account in the calculations according to the Smoluchowski equation [30]. The results are shown in Figs. 9a and 9b. More accurate calculations could be made by applying the soft-particle theory of Ohshima *et al.* [31-33]. However, for the aims of the current study we need mainly the evaluation of the change of ζ. The concrete values are not essential in this case and therefore the Smoluchowski equation gives satisfying results.

Anomalies in the dependences $\zeta = f(C_{ethanol})$ are observed for both live and fixed *E. coli* HB101 cells. Furthermore, these anomalies are in consistent with those in $\gamma = f(C_{ethanol})$ in the concentration region of inactivation (20-40 vol.%), as it is shown in Figs. 10a and 10b. Therefore we could conclude that a reason for the anomalies in $\gamma = f(C_{ethanol})$ at low frequencies are the corresponding anomalies in $\zeta = f(C_{ethanol})$, *i.e.*, in the average electrokinetic electric charge density of the bacteria surface charge of the cells.

At higher frequencies (20 kHz – 20 MHz), which MWP occurs mainly, the reason for the decrease in γ of *E. coli* in presence of ethanol was reported to be different [19] – related to the decrease in the inner electric conductivity of bacteria. However, according to

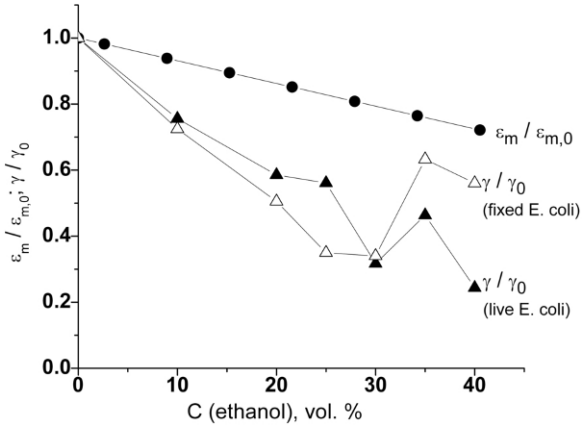

Figure 7. The relative electric polarizability (γ/γ_0) at low frequencies for live and fixed *E. coli* HB101 and the relative dielectric permittivity ($\varepsilon_m/\varepsilon_{m,0}$) of the medium as functions of the concentration of ethanol; γ_0 and $\varepsilon_{m,0}$ are the polarizability γ and the permittivity ε_m at 0% ethanol: γ_0(live) = 4.1 rel. units, γ_0(fixed) = 4.9 rel. units; $\varepsilon_{m,0} = 78\times10^{-12}$ F m^{-1}.

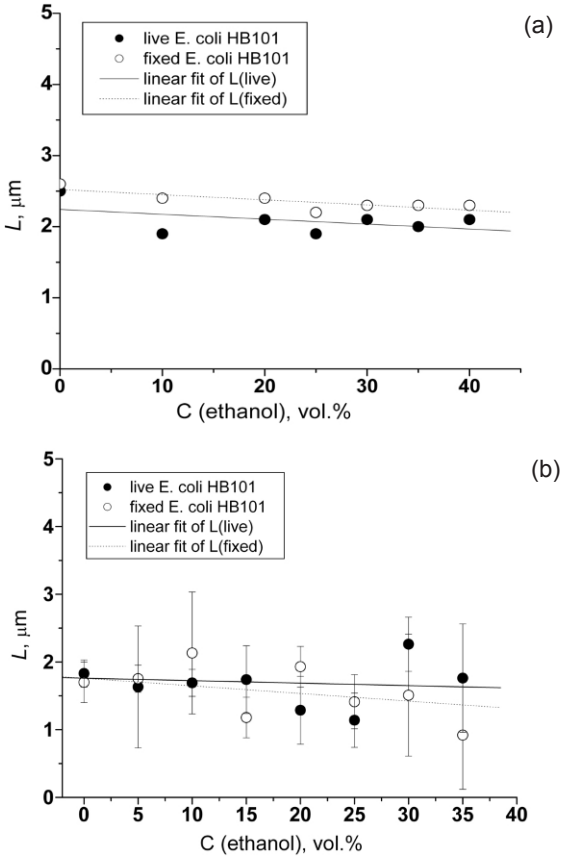

Figure 8. (a) The dependence of the average bacteria length (*L*), determined by electric light scattering (ELS) (Eqs. 2, 3), on the ethanol concentration for live and fixed *E. coli* HB101 cells. The deviations are in the limits of the symbols. (b) The dependence of the average bacteria length (*L*), determined by dynamic light scattering (DLS), on the ethanol concentration for live and fixed *E. coli* HB101 cells.

the extended Maxwell-Wagner theory for the electric birefringence of charged colloid particles by Saville *et al*. [34,35], the MWP of the solid particles depends on their surface electric charge density. The applicability of this theory has not yet been confirmed for the case of bacteria. But there is a slight possibility that the decrease in γ of *E. coli* at higher frequencies in presence of ethanol up to 20 vol.% might be partially related to the decrease in their zeta-potential.

However, there is a discrepancy between the dependencies for the low frequency γ and ζ in Fig. 10a for live *E. coli* HB101 in the lower (<20%) range of ethanol concentration. We do not have an explanation for that deviation. Anyway, it is out of the concentration region of inactivation (20-40 vol.%) and is not very important for the purposes of the current research.

An essential question arises: why does ethanol, which is not electrically charged, change the zeta-potential, related to the surface charge of bacteria?

3.3.4. Molecular mechanism of changing the surface charge of E. coli induced by the non-ionic ethanol

Ethanol changes the properties of solvents. An increase in ethanol concentration reduces the average interactions between the molecules of the solvent, which in turn makes the surface tension of mixed solvents decrease [36]. In the case of water-ethanol solutions, the surface tension drops with 42.11 mN m^{-1} by exponential decay dependence with increasing the ethanol concentration 0-40% [37]. The most significant change in the surface tension (with 34.24 mN m^{-1}) was observed in the concentration range 0-20% and no anomalies were recorded.

It was mentioned that LPS are amphyphilic molecules bound together on the bacterial surface by various forces including electrostatic, hydrophobic interactions and hydrogen bonds [3]. So the bacterial surface could be considered as a LPS-layer with hydrophobic chains anchored in the membrane and hydrophilic heads at the interface of the aqueous medium.

Being this kind of surface active molecule, ethanol would adsorb at the interface. The increasing ethanol content would lead to a reduction in the hydrophobic interactions between the surfactants. This has been proven to result in a decrease in the value of the surfactant saturation adsorption in ethanol-water solution [36]. According to Huang *et al*. [36], there are two possibilities. First, ethanol molecules could occupy the empty positions of the adsorption layer, which is not applicable in the case of bacterial surface (there are not empty positions). The second possibility is that the ethanol molecules may substitute some of the adsorbed

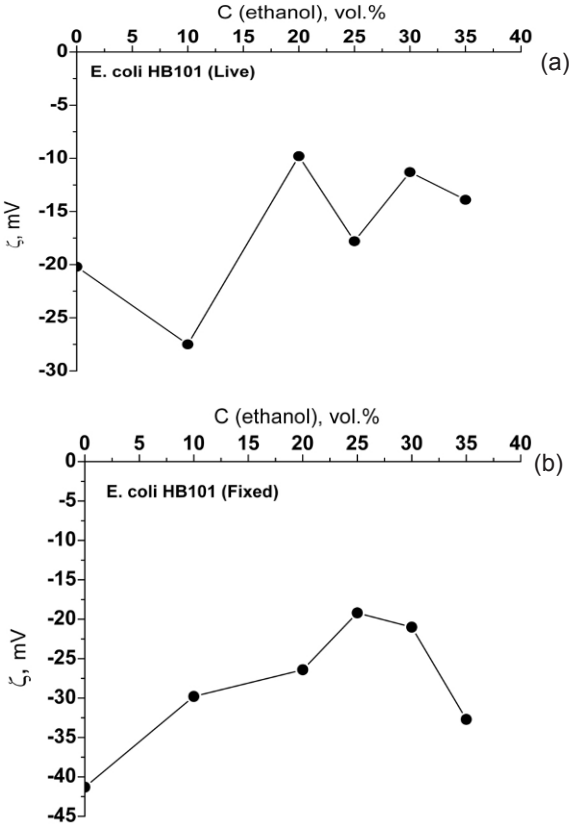

Figure 9. (a) The dependence of the zeta-potential (ζ) of live *E. coli* HB101 on the concentration of ethanol. (b) The dependence of the zeta-potential (ζ) of fixed *E. coli* HB101 on the concentration of ethanol.

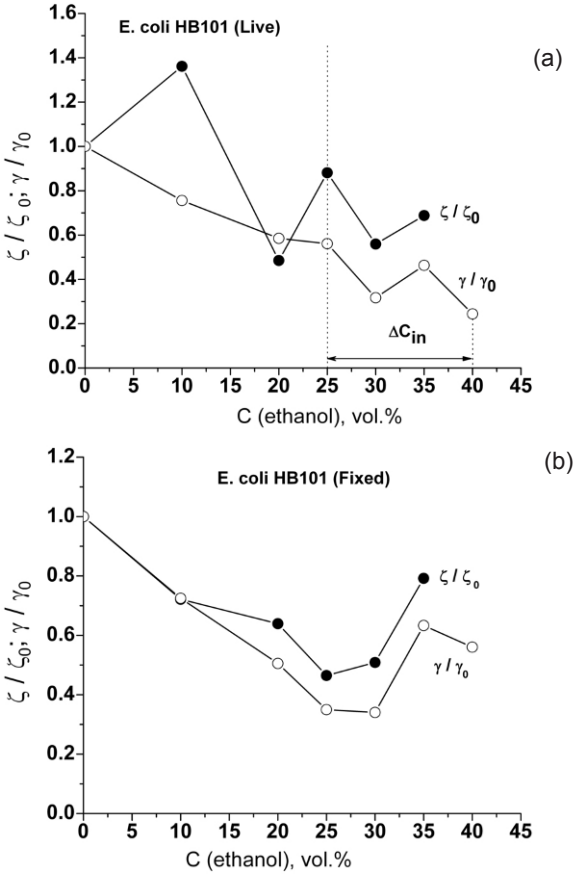

Figure 10. (a) The relative electric polarizability (γ/γ_0) at low frequencies and the relative zeta-potential (ζ/ζ_0) of live *E. coli* HB101 as functions of the ethanol concentration; γ_0 and ζ_0 are the polarizability γ and zeta-potential ζ at 0% ethanol: γ_0(live) = 4.1 rel. units, ζ_0(live) = - 20.2 mV; ΔC_{in} – concentration range of inactivation. (b) The relative electric polarizability (γ/γ_0) at low frequencies and the relative zeta-potential (ζ/ζ_0) of fixed *E. coli* HB101 as functions of the ethanol concentration; γ_0 and ζ_0 are the polarizability γ and zeta-potential ζ at 0% ethanol: γ_0(fixed) = 4.9 rel. units, ζ_0(fixed) = - 41.3 mV.

surfactant molecules. This gives us a base to suggest a hypothesis explaining the molecule mechanism for change in the zeta potential.

As mentioned, the surface electric charge is carried by the LPS molecules. If some of these molecules are released (replaced by ethanol's), the surface charge would decrease because of the non-ionic character of the ethanol molecules. The higher the ethanol content is, higher rate of substitution might be expected. Eventually, this might be related to higher membrane permeability and a decrease in the cytoplasm electric conductivity [19].

It is reported in the literature that some chelating agents such as EDTA cause a decrease of the surface charge due to partial release of LPS from the membrane [38]. Additionally, EDTA is known to increase the membrane permeability [39]. However, EDTA is an ionic compound and the mechanism of interaction with LPS is different than that of ethanol. Further experiments could show if the suggested molecule mechanism is correct.

4. Conclusions

The anomalies in the low frequency electric polarizability γ of *E. coli* HB101 cells as a function of ethanol concentration in the inactivation region (20-40 vol.%) were observed. In contrast, the same dependence is linear at higher frequencies (≥ 20 kHz). Anomalies are recorded for both olive and fixed *E. coli* cells. Therefore, the ethanol inactivation mechanism on these bacteria could be also related to non-biological factors.

In the low frequency range, charge-dependent polarizability (ChDP) occurs. ChDP depends on the medium dielectric permittivity, the average bacteria length, the surface electric charge density (determined by zeta-potential) $\gamma(ChD) = f(\varepsilon_m, L, q)$ and other parameters that remained constant in our experiment.

The decrease in the medium dielectric permittivity in presence of ethanol explains partially only the general decrease in $\gamma = f(C_{ethanol})$ but not the anomalies in the concentration of inactivation. No significant variations in the average length of bacteria are observed in presence of ethanol.

Anomalies in $\zeta = f(C_{ethanol})$ were found. In the concentration range of inactivation, they correlate with those in $\gamma = f(C_{ethanol})$. Therefore, the anomalies in the low frequency polarizability are related to corresponding changes in the surface electric charge density of bacteria.

We suggest a molecular mechanism to explain how the non-ionic ethanol could influence the surface electric charge carried by the LPS molecules. As surface active, ethanol molecule might substitute some LPS molecules (as observed for other surfactant Gibbs layers). This would lead to decrease in the surface charge and eventually to higher membrane permeability because of decreasing the hydrophobic interactions in the surface layer.

However, this can not explain the anomalies in the low frequency polarizability and zeta potential at some particular values of ethanol concentration.

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