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# The Effect of Various Potentials on the Attachment of *Saccharomyces Cerevisiae* and *Staphylococcus Epidermidis* to Carbon Paste Electrodes.

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The attachment process of microbial cells to the carbon paste electrodes with applying various potentials was observed. An electrochemical method was used for the study of the effect of various potentials on the attachment of *Saccharomyces cerevisiae* and *Staphylococcus epidermidis* cells at pH 7.2. The attachment process was analyzed by measuring the electric current derived from the dye (amido black) adsorbed on the vacant areas of carbon paste electrodes (CPE) after attachment of microbial cells. A free attachment (not enhanced by potentials) to the surface of CPE occurred for both species with significantly higher extent for *S. epidermidis*. It was found that the attachment of both microorganisms was significantly enhanced by applying the positive potentials. Particular potentials resulted in higher extent for the adhesion of both S. *cerevisiae* and *S. epidermidis* to the surface of CPE were determined. A probable deliberation of the cells from the surface of CPE occurred when applied -300 mV during attachment of *S. cerevisiae*, and -500 and -600 mV for *S. epidermidis*. Additionally, the time of incubation and viability of the yeast cells influenced their attachment to the surface of carbon paste electrodes.

**Keywords:** Carbon paste; cell attachment; Saccharomyces; Staphylococcus; electrochemical potential

## 1. INTRODUCTION

The surface characteristics of bacterial and yeast cells including hydrophobicity, surface charge and electron donor/acceptor properties play an important role in their attachment to various surfaces. Adherence of microorganisms is accepted as the initial step in the process of colonization of the host tissue or the formation of biofilm [1]. Microorganisms acquire a surface electric charge in aqueous environment due to the ionization of their surface chemical groups, such as amino, carboxyl and phosphoryl groups. The negative charge mostly predominates in bacteria and yeast cells. Since the cell

surface is in direct contact with the environment, the charged groups within the surface of cells may interact with charged ions or molecules present in the external medium [2, 3]. While hydrophobic interactions between hydrophobic molecules on the surface of the microorganism cells and the inert surface predominate at long distance (cca 10 nm), the electric surface charge acted at shorter distances [4].

The surface charge of microorganisms is necessary to determine and give us the information whether the cells may attached on the particular surface. Microbial surface charge is often determined using electrostatic interaction chromatography or by measurement of the electrophoretic motilities, and hence the determination of zeta potential using Smoluchowski equation [5-7]. Alcian blue retention ratio represents another method for the determination of the surface charge of yeast cells. Alcian blue is a type of phthalocyanine complex that has four positive charged sites in the molecule and is adsorbed by the negatively charged yeast cell surfaces [8]. The study demonstrated the electrical detection and characterization of bacterial adhesion onto semiconducting indium tin oxide using the combined hydrodynamic flow system with the electrochemical impedance spectroscopy technique was presented by Bayoudh et al [9]. Morisaki et al [10] proposed a method for study of attachment of bacterial cells to carbon electrodes. The bacterial attachment process was analyzed indirectly by measuring the electric current derived from the organic dye adsorbed on the surface of carbon paste electrodes (CPE). They study the attachment of *Pseudomonas syringe* using anodic stripping method which allowed accumulating bacterial cells to the surface of CPE enhanced by the particular potential previously determined by the measurement of electrophoretic mobility [11]. The study of Morisaki et al [10] was an inspiration for the forthcoming study. The aim of our research is to examine the effect of various potentials on the attachment of microbial cells to the surface of carbon paste electrode via measurement of oxidation current of organic dye (amido black). Since the negative surface charge generally predominates in the surface of microbial cells, the enhanced attachment is excepted if positive potentials are used during accumulation process. We also try to determine if there is a particular potential which significantly enhance the adsorption of microbial cells to the surface of CPE.

## 2. EXPERIMENTAL DESIGN

### *2.1 Organisms and culture condition*

*Saccharomyces cerevisiae* CCM 8191 and *Staphylococcus epidermidis* CCM 4418 (Czech Collection of Microorganisms, Masaryk University, Brno) stock cultures were prepared as follows: the freeze-dried cells of *S. cerevisiae* were activated by transfer into malt extract broth (MALT, HiMedia, Bombay, India) at 30°C for 24 h followed by an appropriate incubation in MALT agar plate. Nutrient agar no. 2 (MPA, HiMedia, Bombay, India) was used for activation of freeze-dried cells of *S. epidermidis* at 37°C for 24 h followed by incubation of appropriate inoculum in MPA agar plate at the same conditions. Both stock cultures were kept in refrigerator and refreshed each 2 weeks.

For the purpose of the study, a fresh culture was prepared from the stock culture by incubating inoculum in freshly prepared MALT (30°C, 24 h) and MPA (37°C, 24 h) agar plate for *S. cerevisiae*

and *S. epidermidis*, respectively. Thereafter, a cell suspension was prepared in phosphate buffer saline (pH 7.2). The concentration of the cell suspension was adjusted to 1.5 according to McFarland turbidimetric standard.

### *2.2 The preparation of carbon paste electrodes*

A mixture of 0.5 g of graphite powder 5.5-7.0 μm (CR-5, Maziva Týn n. L., s.r.o., Czech Republic) and 130 μl of mineral oil (M5904, Sigma-Aldrich, Germany) was prepared in ceramic mortar. Freshly prepared carbon paste was packed into the teflon piston holder with inner diameter of 2.0 mm [12]. The resistance of such prepared electrode was 6.3 ± 0.3 Ω.

### *2.3 Chemicals*

All the reagents were purchased in Sigma-Aldrich (Germany). Amido black dye solution (1.0 μmol $l^{-1}$) was prepared by dilution of appropriate amount of the dye in phosphate buffer (pH 7.2). Distilled water purified by deionized water system was used in this study (G ≤ 0.055 μS). The soluble oxygen was removed from all the solutions by purging with argon for 15 min (purity 99.99%, Linde Technoplyn, Prague, Czech Republic).

### *2.4 Electrochemical measurement*

Three electrode system consisting of carbon paste electrode (working), Ag/AgCl/3.0 M KCl (reference) and platinum wire (counter electrode) connected to PalmSens (Ivium Technologies, Netherland) was used for electrochemical measurement. The surface of CPE was regenerated by renewing and polishing it on wet filter paper before each measurement.

### *2.4 The free adsorption of organic dye and microorganisms to the surface of CPE*

CPE was immersed into 4 ml of 1.0 μmol $l^{-1}$ amido black solution (pH 7.2). Preliminary experiments showed that the oxidation current of the dye at the concentration 1.0 μmol $l^{-1}$ did not change at the stirring speed 400 rpm and after 1.5 min. Therefore, the dye accumulation was performed in 2 min stirring at 400 rpm. The CPE with adsorbed organic dye was immersed in fresh phosphate buffer (pH 7.2) and the oxidation current of amido black ($I_0$) was determined using square wave voltametry (SWV, frequency 25 Hz, potential range from 0 to + 1.0 V, potential step 0.025 V and potential pulse 0.025 V). These $I_0$ values served as blank throughout the whole experiment.

Working electrode was immersed into 4 ml of cell suspension (1.5 x $10^8$ cfu $ml^{-1}$) in phosphate buffer saline (pH 7.2) for 0.5, 1.0, 2.0 and 3.0 min at 400 rpm After the attachment process, amido black solution was allowed to adsorb for 2 min at 400 rpm with the electrode system switched off. CPE with adsorbed cells and organic dye was dipped into fresh phosphate buffer solution (pH 7.2) and the

oxidation current of amido black ($I_{0(cells)}$) was examined using SWV with the condition described above.

The extent of free cell adsorption ($I_{0(extent)}$) to the surface of CPE was calculated using equation:

$$I_{0(extent)} = I_0 - I_{0(cells)} \quad (1)$$

where $I_0$ is the oxidation current of the adsorbed dye (μA), $I_{0(cells)}$ is the oxidation current of the dye adsorbed after accumulation of the cells of microorganisms (μA). The more cells attached to the surface of the electrode the greater the value of $I_{0(extent)}$.

*2.5 The adsorption of microorganisms to the surface of CPE using various potential.*

CPE was immersed into 4 ml of cell suspension (1.5 x $10^8$ cfu $ml^{-1}$) in phosphate buffer (pH 7.2) for 0.5, 1.0, 2.0 and 5.0 min at 400 rpm and various potentials from -600 to +600 mV (100 mV step) were applied. The cell suspension was then changed for amido black solution and the adsorption process continued for 2 min at 400 rpm with the electrode system switched off. The oxidation current ($I_{E(cells)}$) of amido black adsorbed on the surface of CPE was then determined using SVW in phosphate buffer (pH 7.2). The extent of cell adsorption affected by applied potential ($I_{E(extent)}$) was calculated according to equation:

$$I_{E(extent)} = I_0 - I_{E(cells)} \quad (2)$$

The results are interpreted as follow: $I_{0(extent)} \geq I_{E(extent)}$ means that the adsorption of microorganisms was not enhanced by the application of potential, i.e. the amount of the cells on the surface of CPE was similar or lower in comparison with free adsorption of cells. If $I_{0(extent)} < I_{E(extent)}$, the attachment of cells of microorganisms was enhanced by the application of potential.

*2.6 Statistical analysis.*

The experiments were carried out in three separate trials and individual measurements were replicated five times at least. The results are expressed as average values with standard deviations. Analysis of variance was used for determination of statistical differences at the probability level p = 0.05.

## 3. RESULTS

*3.1 The free adsorption of organic dye and microorganisms to the surface*

Anodic signal for oxidizing the adsorbed dye appeared at 670 mV vs. Ag/AgCl. When yeast or bacterial cells were attached to the surface of the electrode surface before adsorbing the dye, the oxidation current was smaller compared with the case where no cells were attached (Figure 1).

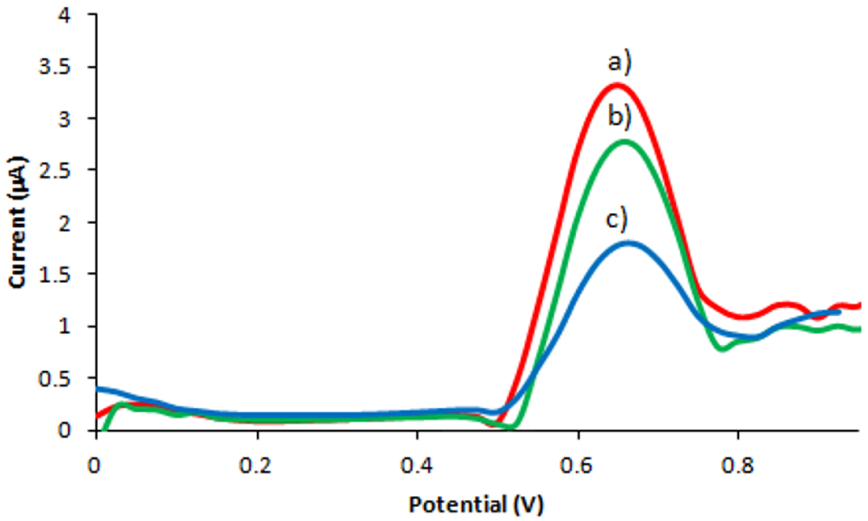


**Figure 1.** Square wave voltammograms of amido black (1.0 µmol $l^{-1}$) adsorbed on CPE. (a) No microbial cells attached resulting current $I_0$, (b) CPE allowed to attach bacterial cells for 1 min stirring at 400 rpm ($I_{0(cells)}$) and (3) CPE attached microbial cells for 1 min (400 rpm) using potential 400 mV ($I_{E(cells)}$).

It was found that the time of adsorption had an effect on the amount of the accumulated cells of *S. cerevisiae* cells up to 1.0 min. As shown in Fig. 2, the oxidation current of dye adsorbed in 0.5 min on the surface of CPE was significantly lower than those determined in 1.0 min of accumulation ($p < 0.001$). Further increase of accumulation time had no significant effect on the attachment of *S. cerevisiae* cells. The same relationship has been determined for *S. epidermidis* (not shown).

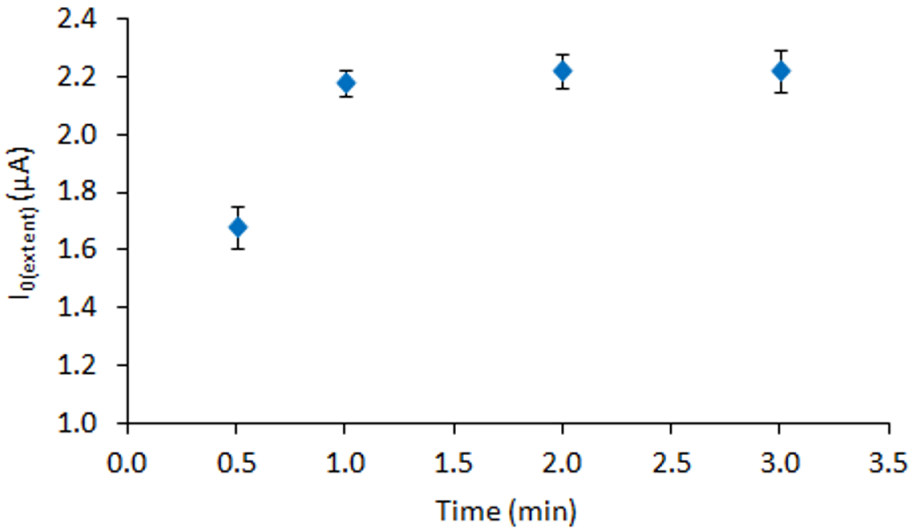


**Figure 2.** Increase in the anodic peak current of amido black along with the attachment time of yeast cells. Mean values with standard deviations (n = 20).

Microbial cells attachment process was described by the $I_{0(extent)}$ values in this study. The accumulation of *S. cerevisiae* resulted in 0.74-0.76 µA whereas *S. epidermidis* gave higher values 1.816-1.818 µA., i.e. *S. epidermidis* cells occupied more spaces in the surface of carbon paste electrode (Table 1). It should be also noted here that values of oxidation current of the dye varied in different CPE; however the differences ($I_{0(extent)}$) are reproducible (Table 1).

**Table 1.** The oxidation current of 1.0 µmol $l^{-1}$ amido black in phosphate buffer (pH 7.2) before and after of free attachment of *Saccharomyces cerevisiae* and *Staphylococcus epidermidis* to the surface of carbon paste electrode (n = 15).

| No. of CPE | $I_0$ (µA)[1] | $I_{0(cells)}$ (µA)[2] | $I_{0(extent)}$ (µA)[3] |
|---|---|---|---|
| | *Saccharomyces cervisiae* | | |
| 3 | 2.99 ±0.06 | 2.22 ± 0.02 | 0.76 ± 0.06 |
| 6 | 3.54 ± 0.08 | 2.80 ± 0.05 | 0.74 ± 0.08 |
| | *Staphylococcus epidermidis* | | |
| 4 | 3.17 ± 0.07 | 1.35 ± 0.02 | 1.82 ± 0.08 |
| 5 | 5.42 ± 0.03 | 3.55 ± 0.01 | 1.82 ± 0.03 |

[1] $I_0$ the oxidation current of amido black after 2.0 min accumulation (stirring speed 400 rpm); [2] $I_{0(cells)}$ the oxidation current of amido black after free attachment of microbial cell; [3] $I_{0(extent)}$ the extent of free cells adsorption (eq. 1).

*3.2 The adsorption of microorganisms on the surface of CPE using various potential.*

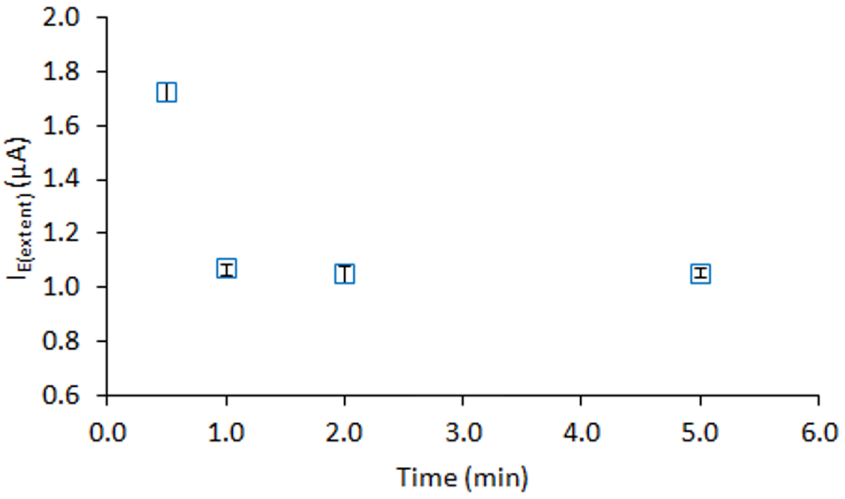


**Figure 3.** Decrease in the anodic peak current of the dye (amido black) along with attachment time of yeast cells. Mean values with standard deviations (n = 15).

When particular potential enhanced the attachment of the cells to the surface of the carbon paste electrode, the oxidation current of the dye (amido black) decreased in comparison with that

obtained after free attachment of the cells (Figure 1). Applying the potential, the amount of cells accumulated to the surface of CPE depended on the time of accumulation. For instance, the oxidation current peak decreased after 0.5 min of accumulation of *S. cerevisiae* cells at 400 mV followed by steady values from 1.0 to 5.0 min (Figure 3). The similar relationships were observed in other potentials in both *S. cerevisiae* and *S. epidermidis* cells.

This indicated that the microbial cells reached to adsorption equilibrium after 1.0 min. Figure 4 shows the effect of applied potential on the attachment of *S. cerevisiae* on the surface of carbon paste electrode. According to the results of previous studies [7, 13] where negative surface charges have been determined, the values of $I_{E(extent)}$ for positive potential range 100-600 mV were significantly higher ($p < 0.001$) in comparison with free cells adsorption ($I_{0(extent)}$). When potentials 100, 300 and 400 mV were applied, the greatest values of $I_{E(extent)}$ were obtained. It means that the surface of yeast cells was negatively charged and the potentials -100, -300 and -400 mV predominated on their surface. In negative potential range, the $I_{E(extent)}$ values were similar to that after free adsorption process ($p > 0.05$) except of the potential -300 mV. Applying that negative potential, the opposite effect was determined. The cells were probably deliberated from the surface of the CPE, thus more organic dye accumulated to the surface of working electrode and the smaller $I_{E(extent)}$ was obtained.

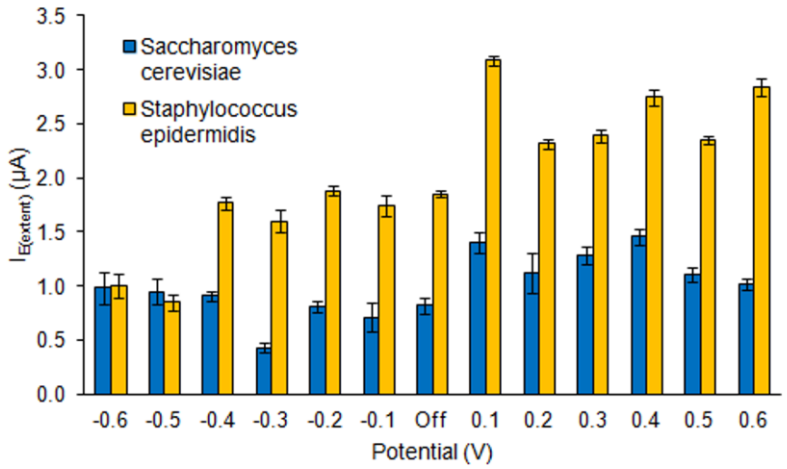


**Figure 4.** The anodic peak currents of the dye (amido black) after attachment of *Saccharomyces cerevisiae* (blank columns) and *Staphylococcus epidermidis* (grey columns) affected by electrochemical potentials for 1 min (400 rpm stirring). Off, the free attachment process (with electrode system switched off). Mean values and standard deviations (n = 15-20).

The positive potentials applied during accumulation of bacteria *S. epidermidis* enhanced their adsorption to the surface of CPE in comparison with that occurred during free adsorption process (Figure 4), i.e. the surfaces of *S. epidermidis* are negatively charged ($p < 0.001$). The attachment of bacterial cells to the surface of CPE was promoted at the potential 100 mV followed by the potentials 400 and 600 mV. While the negative potentials from -100 to -400 mV had no effect on the adsorption

of *S. epidermidis* cells, a smaller values of $I_{E(extent)}$ have been observed during accumulation at -500 and -600 mV probably resulting from the decreased amount of cells attached to the surface of CPE.

We also tried to examine the effect of the age of the culture on the extent of microbial cells attachment process ($I_{E(extent)}$) and whether the adsorption process can be affected by viability of the cells. A culture of *S. cerevisiae* incubating at 30°C for 7 days was allowed to attach to the surface of CPE with applied potential 400 mV (1.0 min, 400 rpm). After subsequent adsorption of organic dye (2.0 min, 400 rpm), the oxidation current was determined resulted in $I_{E(extent)}$ value of 1.52 ± 0.04 μA, which was significantly lower in comparison with the $I_{E(extent)}$ value obtained for 24 h culture of *S. cerevisiae* (1.46 ± 0.09 μA, $p < 0.01$). The same experiment performed with inactivated cells of *S. cerevisiae* (15 min inactivation at 100°C) revealed that the attachment process of dead yeast cells was also enhanced by applied potential 0.4 V resulting in $I_{E(extent)}$ = 1.66 ± 0.04 μA, which was significantly higher ($p < 0.001$) than those obtained for living cells.

## 4. DISCUSSION

In this research, the attachment process of both yeast and bacterial cells to the surface of CPE was observed during magnetic stirring without applying the potentials. Although the adsorption process reached to its equilibrium, it was previously observed that the cells did not cover the entire surface of CPE [10]. The size of the cells probably plays an important role in attachment of these microorganisms. The size of *S. epidermidis* cells are smaller (0.5-1.5 μm) that in *S. cerevisiae* (5-10 μm), therefore the smaller cells could easily migrate into the pores of the carbon paste composite material (Table 1). Although we did not observe the microscopic characteristics of the prepared carbon paste, the study of Švancara et al [14] showed that carbon paste prepared with multicrystalline carbon powder (5-10 μm) exhibited a complicated structure formed by "carbon chips" of various shapes and sizes. An interesting work about the effect of the disordered hemispherical micropatterns on *S. epidermidis* retention and biofilm formation on silicone substrate has been published [15]. The authors found that the size of structured patterns is an important factor in altering bacteria adhesion. The bacteria initially adhered on the unpatterned areas of the disordered surface. We may here hypothesize that the smaller particles (i.e. *S. epidermidis* cells) could better attach to such a highly structured surface.

The differences in the extent of attachment for yeast and bacteria should be also linked with various cell wall compositions. Cell walls of yeast are made of a microfibrillar array of β 1-3 glucan, overlaid by β 1-6 glucan and mannoproteins, a highly glycosylated polypetides. An important minor component is chitin, a linear polysaccharide of β 1-4 linked N-acetylglucosmaine [16]. The recent study of the structure of *S. cerevisiae* cell walls by atomic force microscopy showed that the polypeptides were more exposed and available for interaction in comparison with other brewery yeast strain [17]. The negative charge of the cell wall of yeast resulted from phosphate, pyruvate or glucuronic acid which can be linked to mannoproteins [18]. The attachment of *S. cerevisiae* cells was significantly enhanced by the application of positive potentials in our study indicating that the surface of the yeast cells is negatively charged. Moreover, concrete electrochemical potentials which improved

the attachment process of yeast cells were identified. On the other hand, Rapoport et al [19] found that higher amounts of yeast cells were immobilized on the surface of hydroxyapatite ceramic tablets with higher negative charges probably due to positively charged groups of mannoproteins. Different yeast strains or various culture conditions can be responsible for such a discrepancy. The surface of gram-positive bacteria has a peptidoglycan layer on which teichoic acid, teichunoric acid, and proteins are covalently bound bearing the negative charge [20]. On the other hand, *Staphylococcus* strains also had some positive charged groups in membrane which allowed them to connect with negatively charged nanoparticles [21]. *S. epidermidis* did not exhibit positively charged in our study where the amount of the cells adsorbed to the surface of CPE appeared to be similar to that during free accumulation process. The cause of the deliberation of both yeast and bacterial cells at particular negative potential is not clear, however these findings seem to be interesting and need to be examine more extensively in future. The electrochemical polarization of microbial cells already attached to the surface of CPE may play a certain role [22].

The results suggested that morphological changes occurred in cell walls during prolonged incubation and after inactivation of yeast cells and more negatively charged groups were exposed to the environment. Our results are in agreement with the previous study of Vichi et al [7] who found that *S. cerevisiae* changed its cell surface properties during sparkling wine ageing. Klodzinska et al [23] suggested that the surface charge of bacterial cells also vary depending on its physiological state.

In conclusion, particular electrochemical potentials enhancing the attachment process of microbial cells were determined in this study. Both *S. cerevisiae* and *S. epidermidis* were negatively charged, i.e. enhanced attachment was observed when positive potentials were applied. Among these positive potentials, particular ones supported the attachment more than the other. While the potentials 100, 300 and 400 mV enhanced the attachment of *S. cerevisiae*, 100, 400 and 600 mV supported the adsorption of *S. epidermidis* cells to the surface of carbon paste electrodes. Although the cell wall composition differ in yeast and bacteria, the attachment of both *S. cerevisiae* and *S. epidermidis* was enhanced by the same potentials at pH 7.2 (100 and 400 mV). Surprising data have been obtained when negative potentials were applied during attachment process. A significantly higher amount of dye (i.e. lower amount of microbial cells) was adsorbed after attachment of yeast using potential -300 mV and after attachment of gram-positive bacteria using potentials -500 and -600 mV. Since we did not find any significant clarification of this behavior in literature (Scopus database), a further study needs to be carried out. Moreover, the electrochemical method presented in this study seems to be promising for the study of electrostatic-based adsorption mechanism of microbial cells.

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