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Application of electrochemical optical waveguide lightmode spectroscopy for studying the effect of different stress factors on lactic acid bacteria☆

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Abstract

Electrochemical optical waveguide lightmode spectroscopy (EC-OWLS) has been developed to combine evanescent-field optical sensing with electrochemical control of surface adsorption processes. For bioanalytical sensing, a layer of indium tin oxide (ITO) served as both a high-refractive index waveguide and a conductive electrode. In addition, an electrochemical flow-through fluid cuvette was applied, which incorporated working, reference, and counter electrodes, and was compatible with the constraints of optical sensing.

The subject of our study was to monitor how the different stress factors (lactic acid, acetic acid and hydrogen peroxide) influence the survival of lactic acid bacteria. The advantage of EC-OWLS technique is that we could carry out kinetic studies on the behaviour of bacteria under stress conditions, and after exposure of lactobacilli to acid and oxidative stress we get faster results about the status of bacteria compared to the traditional quantitative methods.

After optimization of the polarization potential used, calibration curve was determined and the sensor response of different rate of living and damaged cells was studied. The bacterial cells were adsorbed in native form on the surface of the sensor by ensuring polarizing potential (1 V) and were exposed to different concentration of acetic acid and hydrogen peroxide solution to 1 h, respectively and the behaviour of bacteria was monitored. Results were compared to traditional micro-assay method.

Keywords: Electrochemical optical waveguide lightmode spectroscopy (EC-OWLS); Lactic acid bacteria; Indium tin oxide (ITO) covered sensor; Real-time measurement; Stress factors

1. Introduction

In the field of biotechnological research there is an increasing demand for novel sensor techniques, which offer the possibility for quick, real time kinetic studies. A new sensor system has been developed that combines evanescent-field optical sensing with electrochemical control of surface adsorption processes. This new technique, termed "Electrochemical Optical Waveguide Lightmode Spectroscopy" (EC-OWLS), proved to be efficient in monitoring molecular surface adsorption and layer thickness changes of an adsorbed layer examined in situ as a function of potential applied to a waveguide [1,2]. OWLS technique is based on the precise measurement of the resonance angle of polarized laser light, diffracted by a grating and incoupled into a thin waveguide. Such incoupling resonance occurs at very precise angles depending on the optical parameters of the sensor chips and the complex refractive index of the covering sample medium. The refractive index, determined from the resonance incoupling angle detected at high accuracy, allows the determination of layer thickness and coverage (or mass) of the adsorbed or bound material with ultra high sensitivity [3–5].

Continuously measuring the shift of the incoupling angles allows the direct on-line monitoring of the adsorption of macromolecules above the grating without the need for any labeling procedure. The method is highly sensitive (i.e., detection limits <1 ng cm^{-2}) up to a distance of a few hundred nanometers above the surface of the waveguide. Furthermore, a measurement time resolution of seconds permits an in situ, real-time study of adsorption kinetics. From the two measured parameters two further parameters, the thickness (d_A) and the refractive

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index (n_A) of the adsorbed layer can be simultaneously determined using the mode equations. The absolute values for the surface adsorbed mass density then can be directly calculated from the thickness and refractive index values according to the Feijter's formula. This is very useful especially when the density (thus the refractive index as well) of the adsorbed layer changes during adsorption (i.e., due to hydration, denaturation, etc.) [1].

For the quantification of the attached molecules on the sensor surface different models were investigated depending on the type of the molecules.

The structural evolution of protein monolayers was followed by OWLS technique where the measured effective refractive indices were interpreted using the uniform thin film model. Ramsden [6,7] studied the deposition of protein molecules successfully and concluded, that the mass (M) of protein deposited per unit area could be calculated from the thickness and refractive index of the thin film of deposited molecules. Guemouri and coworkers [8] investigated transferrin and fibronectin molecules for studying how geometric thickness and refractive index evolve as a particle monolayer builds up. For both proteins, since the rate of adsorption constantly diminished and the amount of adsorbed material appeared to approach a plateau, it was inferred that at most a monolayer of protein could be adsorbed. The values of M at saturation calculated from the mode spectrum at the end of adsorption corroborated this inference. Ramsden [9] showed the adsorption property of different forms of poly-L-lysine molecules and pointed out the change in adsorption behaviour with the different conformation. The real time digestion of poly-L-lysine using trypsine was also demonstrated and concluded from the decrease of the calculated mass of the deposited layer on the waveguide surface. Guemouri [10] studied the effect of both low and high salt content on the adsorption of protein fibronectin molecules at surfaces in situ and in real time. The kinetics of the evolution of the adsorbed layer thickness, its packing density, and of the total amount deposited were analyzed and used to deduce that at low ionic strength the protein has a compact conformation prone to lateral clustering at the surface, and at high ionic strength it is in a random extended conformation. Höök and coworkers [11] demonstrated that the adsorption kinetics of model proteins with optical waveguide lightmode spectroscopy (OWLS) and ellipsometry (ELM) provide in most cases consistent and comparable results, which can be straightforwardly converted to adsorbed protein molar mass.

Ramsden and co-workers [12] applied an OWLS method for accurately parametrizing the number and the shape of spreading cells. For many types of cells it has been observed that when a cell suspension is seeded onto a surface the cells will initially attach and subsequently reorganization occurs that results in a flattening and spreading of the cell body. This implies that at the initial point of contact a small flat region forms, whose area increases. It was concluded, that only the shape of cells were changed, the number of cells remained constant. The change in the measured signal was because of the flattening and spreading of the cells on the sensor surface within a short time.

The optical set-up is typical for adsorption and binding studies of biomolecules, but the voltage dependence is a new phenomenon. Previous applications employed waveguiding films of non-conducting materials such as silicon titanium oxide (STO). Indium-tin-oxide (ITO) has been reported as a well-defined optically transparent and electrically conducting layer on different electrode or sensor surfaces. Applied voltages, positive at the ITO surface, shifted the incoupling angle of linearly polarized laser light. Voltages below 1 V are sufficient, while above the effect saturates. Weak dependence on solution composition and strong asymmetry with respect to the polarity of the applied potential imply that the ITO itself is involved in the effect [13].

An applied dc voltage offers a means of controlling immobilization during biosensor fabrication and detection during biosensing application. Brusatori and coworkers [2] presented a method to directly and continuously measure the adsorption of biomacromolecules or other polyelectrolytes, under an applied potential difference, based on OWLS technique. They concluded, that the adsorption of human serum albumin molecules and horse heart cytochrome c molecules considerably enhanced in the presence of an applied potential exceeding 1 V. ITO-covered waveguides incorporating a grating and immersed in a fluid have been used to study the incoupling of light as a function of voltage applied between the ITO and an electrode parallel to the waveguide. Applied voltages, positive at the ITO surface, shifted the incoupling angle of linearly polarized laser light. Applying positive potentials resulted in increased adsorbed mass, presumably due to polymer chain extension and reorganization in the molecular adlayer [14].

In our investigation the EC-OWLS technique as a novel rapid method was applied, which combines evanescent-field optical sensing with electrochemical control of surface adsorption process for the study of stress effect of lactic acid bacteria (LAB) cells.

Lactic acid bacteria are part of the normal human microflora and play an important role in the fermentation of different food products, such as milk, cheese or meats. During the fermentation process lactobacilli are confronted with many inhibitor factors, such as high salt content, temperature changes, organic acids. The major fermentation product of lactobacilli is lactic acid, which has preservative effect through limiting the growth of many bacteria, yeast and moulds in the food product. At low pH a large amount of lactic acid is in undissociated form so it can diffuse passively across the cell membrane, collapse the electrochemical proton gradient, and alter the membrane permeability, thereby leading to the disruption of the substrate transport system [15,16]. Acetic acid acts synergistically with lactic acid; while lactic acid decreases the pH of the medium, thereby increasing the toxicity of acetic acid [17]. Lactobacilli can produce hydrogen peroxide in the presence of oxygen and at molecular level the hydrogen peroxide can react with cellular targets, such as proteins and nucleic acids. Hydrogen peroxide causes the peroxidation of the membrane lipids and alters the membrane proteins, hence it affects the cell permeability and osmoregulation [18].

As it is obvious from the above-mentioned insights, it is of great importance to study the effect of different stress factors on the microbial status of lactic acid bacteria, their growth, viability and survival. The conventionally used methods, such as the plate count technique and the acidification assay [19], are quite reli-