Application Note #000400

KeyWords
XPS, E. coli, Bacteria, Biofilms, Measurements, Surface Analysis

EnviroESCA™

Near-ambient pressure XPS of hydrated *Escherichia coli* samples with EnviroESCA

This application note presents how EnviroESCA can be used to analyze *E. coli* biofilms on silicon under near ambient pressure conditions in various states of hydration. Such investigations of the outer bacterial cell surface in their hydrated state are essential for studying biological interfaces at work.

Motivation

XPS is a very powerful surface analysis technique providing elemental and chemical information from the first 10 nm of a surface, which is fitting quite well the thickness of the outer cell membrane of gram-negative bacteria. Conventional XPS is an ultra-high vacuum technique making it incompatible to bacterial samples which inherently are in a hydrated state. Thus, near-ambient pressure (NAP) XPS is the method of choice when studying biological samples as *E. coli* biofilms.

NAP XPS has been used extensively to study electrochemical and catalytic processes but also bacteria and biofilms can be characterized in various gas environments with minimal sample preparation. Therefore, in situ measurements and studies of biofilm-substrate interactions are easily done.

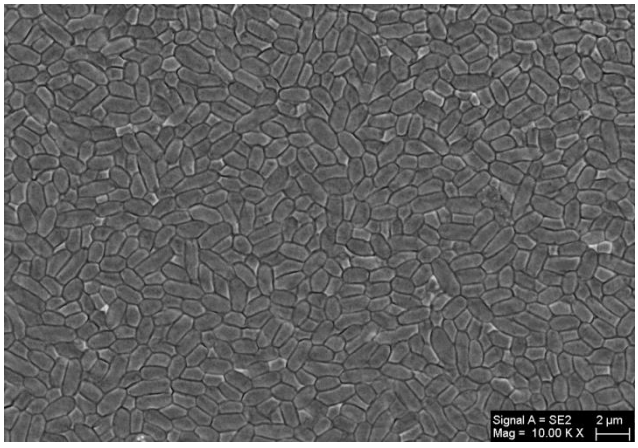

Fig. 1 SEM picture of *E. coli* on silicon at 10,000x magnification

Method

EnviroESCA utilizes X-ray Photoelectron Spectroscopy (XPS) as analytical technique. Here an electron beam is generated inside the X-ray source and focused on an aluminum X-ray anode. The deceleration of the electrons on the anode generates X-rays. This X-ray beam is monochromated and focused on the sample.

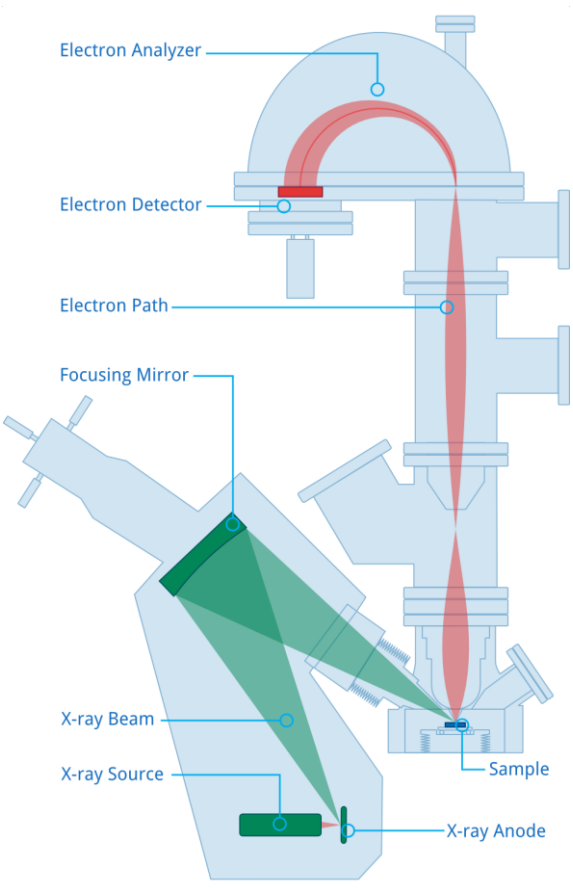

Fig. 2 XPS with EnviroESCA

X-ray photons impinging the sample excite electrons in the material which are subsequently emitted with a specific kinetic energy determined by their binding energy and the photon energy of the X-rays. In case of solid samples only electrons from atoms down to a depth of about 10 nm are able to leave the surface.

These electrons propagate through the lens system of the electron analyzer into the hemisphere which acts as a spherical capacitor forcing the electrons onto circular paths with radii depending on their kinetic energy. The path of photoelectrons ends at an electron sensitive detector where the electrons are amplified and measured as intensity in counts per second. A photoelectron spectrum is recorded by sweeping the voltage of the spherical capacitor while measuring the number of electrons per second on the detector. From these spectra a quantitative analysis of the atomic composition of the sample surface can be done.

Experimental Section

EnviroESCA can work under vacuum as well as near ambient pressure (NAP) conditions up to several dozens of mbar. Thus, it is very well suited to investigate surfaces of hydrated biological samples at elevated pressures.

In EnviroESCA an intrinsic charge compensation which we call Environmental Charge Compensation makes additional low energy electron or ion sources unnecessary. As shown schematically in Fig. 3 illumination of the surrounding gas atmosphere with soft X-rays delivers all the free charges that are needed to compensate for surface charging on the sample.

The NAP-XPS capability of EnviroESCA allows in situ surface studies of a multitude of biological samples in very different environments. Here we present results of a surface chemical analysis of *E. coli* biofilms on silicon using NAP-XPS, for sample preparation see [1].

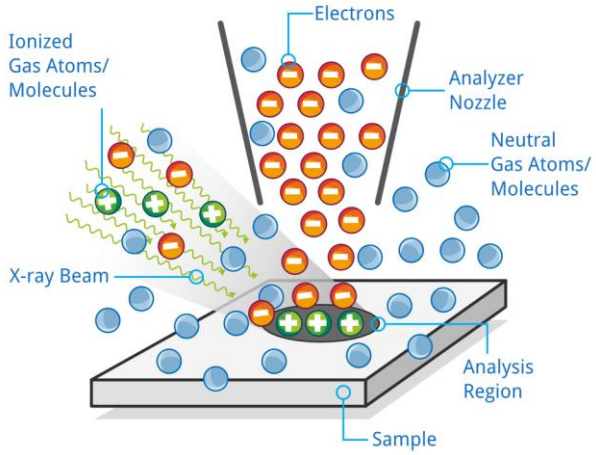

Fig. 3 Environmental Charge Compensation

At first an *E. coli* sample was immediately - after rinsing with buffer solution - transferred into the EnviroESCA and characterized in humidity at 11 mbar. These are conditions where the bacteria are close to their natural hydrated state. The biofilm was then gradually dried in the chamber and measured at 2 mbar.

A second sample was air dried first, introduced into the EnviroESCA, then measured at 4 mbar of ambient air, and after staying in vacuum (10^{-4} mbar) overnight measured again at 1 mbar. That sequence was done to examine how drying influences the surface of *E. coli* biofilms.

Results

The surface of *Escherichia coli* adhered to silicon wafers has been characterized under various conditions: i) humid at 11 mbar, ii) after drying at 2 mbar, iii) pre-dried at 4 mbar, and iv) at 1 mbar after vacuum storage (10^{-4} mbar) overnight.

According to earlier reports on freeze-dried and fast-frozen bacteria obtained with UHV XPS the information from core-level peaks of oxygen, carbon and nitrogen make it possible to differentiate between major cell wall components related to polysaccharides, lipids and proteins/peptides.

Here only carbon C 1s core-level spectra are presented and discussed.

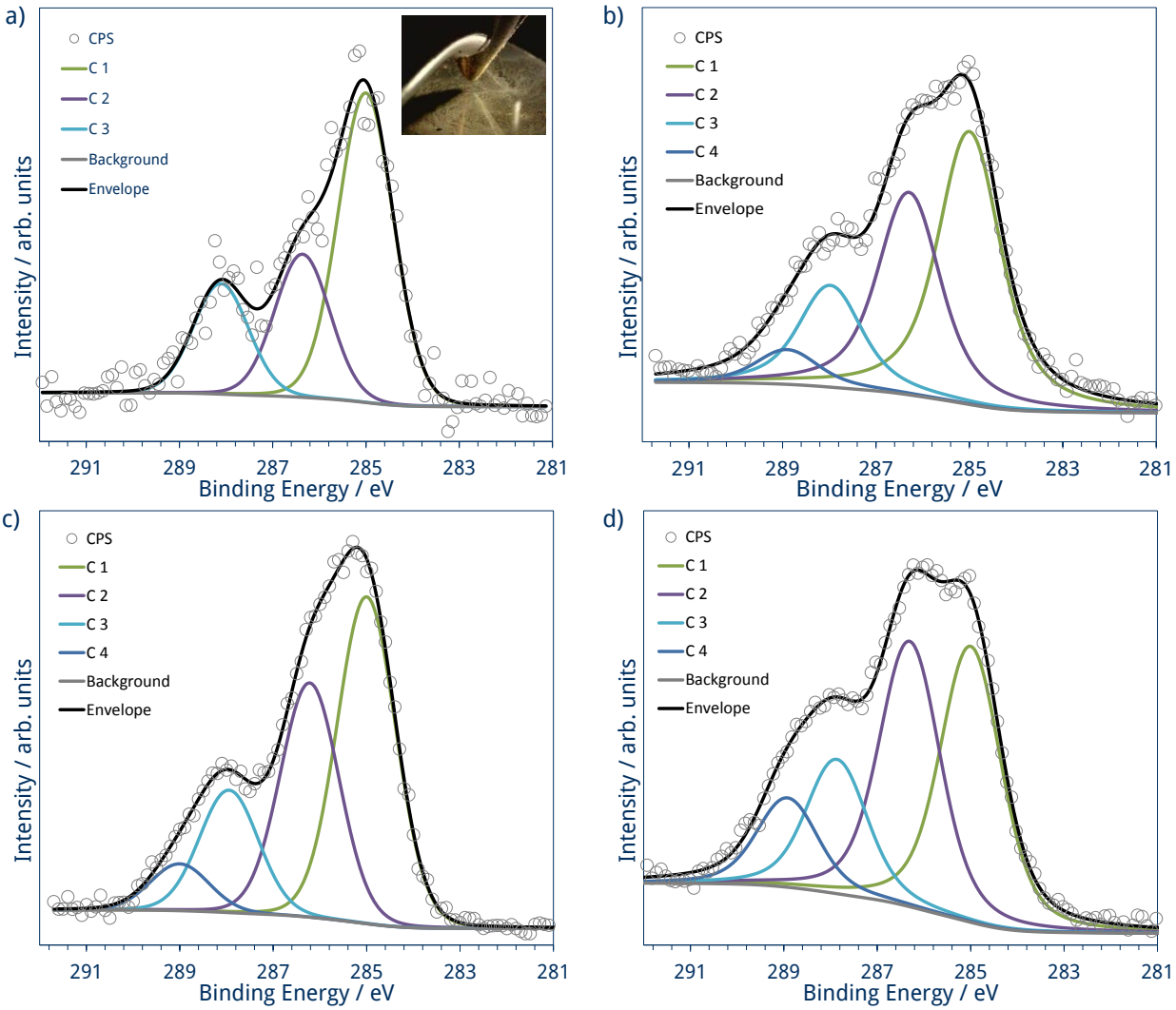

Fig. 4 C 1s core-level spectra of *Escherichia coli* biofilms on Si a) humid at 11 mbar of water vapor, b) dry at 4 mbar, c) dry at 2 mbar, and d) dry at 1 mbar after vacuum storage (10^{-4} mbar) overnight. All spectra are normalized to C 1s maximum intensity.

Figure 4 shows C 1s high-resolution spectra of *E. coli* biofilms on silicon obtained at various states of drying.

The C 1s spectra were fitted with 4 components. Component C 1 is attributed to aliphatic carbon (C-C/C-H) at 285.0 eV, C 2 is assigned to carbon single bonded to oxygen or nitrogen from polysaccharides or peptides/proteins (C-O, C-N), C 3 originates from carbonyl (C=O), amide (O=C-N) or acetal (O-C-O) groups, and C 4 is related to carboxylic acids (COOH) or carboxylate esters (COOR). A comparison of the peak components reveals that C 2, C 3, and C 4 increase relatively to the aliphatic carbon C 1 as the pressure decreases. After overnight drying, the C 1 and C 2 components have approximately the same peak area, and the carboxylic carbon C 4 has its largest peak area, while it was not present at all under humid conditions (cf. Fig. 4).

Additionally one has to note the lower signal-to-noise ratio at 11 mbar compared to the other pressures, which is due to increased electron scattering in the gas atmosphere at higher pressure and therefore an overall lower count-rate results.

Beam damage has to be considered for any XPS analysis of biological samples. Therefore, C 1s spectra have been taken at the beginning and at the end of a sample's measurement series. However, no notable changes were observed in the C 1s spectra of all the investigated bacterial samples.

Conclusion

EnviroESCA with its ability to work in near-ambient pressure conditions using different gas atmospheres allows *in situ* surface characterization of real bio samples as bacteria, cells, biofilms or viruses under biological relevant conditions. Bacteria and biofilms can be grown directly on the chosen substrate under controlled conditions, and studied in near-ambient conditions with minimal alteration and pre-treatment of the sample.

Different samples of *E. coli* adhered to silicon substrates have been characterized by NAP-XPS in various humid and dry states. Those measurements demonstrated the unique capability of EnviroESCA to analyze surfaces of bacterial samples during drying.

Substantial changes in the C 1s peak attributed to an increased signal from polysaccharides could be observed during dehydration. It was found that the amount of carbon components associated with polysaccharides increases relative to aliphatic carbon during drying and increases further after overnight storage in vacuum, which implies a significant impact of the drying on the bacterial surface.

[1] Kjærvik M, Schwibbert K, Dietrich P, Thissen A, Unger WES. Surface characterisation of *Escherichia coli* under various conditions by near-ambient pressure XPS. *Surf Interface Anal.* **2018**, 1–5. https://doi.org/10.1002/sia.6480

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