Characterisation of proteins-like and humic-like substances fraction from biological sludges exopolymeric substances by HPLC-SEC using fluorescence detector

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EPS from biofilm or biological sludges are a complex mixture of high molecular weight macromolecules with ill-defined structures, variable molecular size (MS) and chemical properties. EPS contains polysaccharides, proteins and humic-like substances as major constituents (Wingender et al., 1999; D'abzac et al., 2010) and nucleic acids, uronic acid and phospholipids are the minor constituents (Frolund et al., 1996). The molecular masses of EPS lie from a range of few thousands to several million Daltons (Simon et al., 2009). Many colorimetric methods have been used to characterize EPS. These characterizations of EPS give only quantitative information *i.e*. the total concentration of polysaccharides, proteins, humic-like substances, uronic acids, nucleic acids, etc. (Wingender et al., 1999; Liu and Fang, 2003). For better characterization, qualitative informations are required. Size exclusion chromatography (SEC) can give access to valuable information about the fingerprint and/or the distribution of apparent molecular weight of EPS present in sludge (Frølund and Keiding, 1994; Görner et al., 2003; Comte et al., 2007).

In this study, EPS fingerprint from activated or anaerobic granular sludges obtain by size exclusion chromatography (SEC) were recorded with UV and fluorescence detection. For this purpose, a combination diode array detector and fluorescence detector was used to record EPS chromatograms. The results of recorded fingerprints at wavelength 210 nm and 280 nm with diode array detector were considered. Similar wavelengths, 210 nm and 280 nm, were also mentioned in the several other studies (Görner et al., 2003; Comte et al., 2007; Simon et al., 2009). In the literature, the fingerprints obtained at 210 nm corresponds for the aliphatic-like compounds such as polysaccharides or uronic acid or mineral nano-particles, whereas the EPS fingerprints obtained at a wavelength of 280 nm, likely correspond to conjugated molecules such as proteins, humic-like substances or nucleic acid (Görner et al., 2003; Garnier et al., 2006). The couple of wavelengths which were selected with fluorescence detection was carried by Excitation–emission matrix (EEM) fluorescence spectroscopy. The fluorescence spectra showed the presence of protein-derived compounds and humic-like substances in investigated EPS samples. The chosen peak maxima locations of excitation-emission wavelengths were 221/350 nm for protein-like substances whereas, for humic-like substances 345/443 nm for EPS from activated sludge samples, 335/458 nm and 345/443 nm for anaerobic granular sludge. According to literature, a wavelength of 280 nm is more selective for proteins than a wavelength of 210 nm to record chromatograms due to the absorbance of conjugated compounds at this wavelength (Simon et al., 2009). In this study, the proteins fingerprints of EPS, recorded at 280 nm, change dramatically, mainly in the relative absorbance of peaks when they were measured by a specific florescence EEM of proteins-like molecules.

Concluded Results

- Fluorescence detector enables a better and more specific characterization of protein and humic-like substances fraction than diode array detector at wavelength 210 and 280 nm.
- The protein and humic-like substances of EPS extracted from sludge are qualitatively characterized on the basis of apparent MW and their fluorescence intensity.
- EPS display a protein-like fraction with a wide range of apparent MW (>600KDa and 439 - 0.8KDa).
- The humic-like substances fraction from EPS present molecules with a low apparent MW (100 - 0.8KDa).
- The origin or types of sludge and extraction procedure affect the fingerprints of both protein-like and humic-like substances.

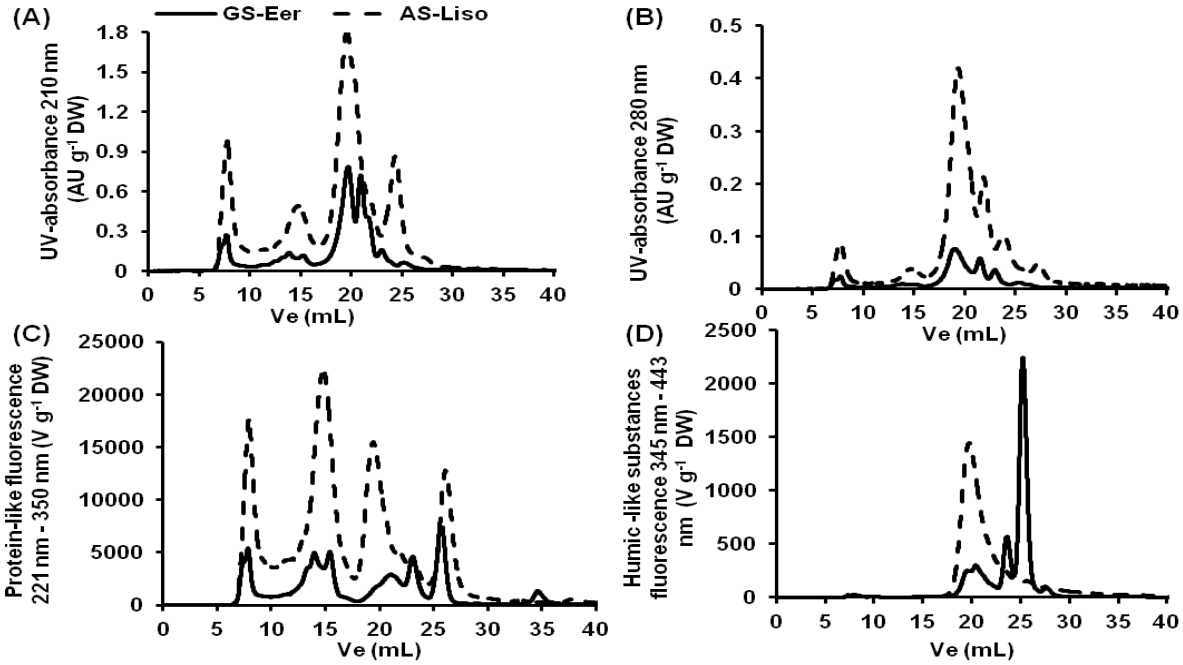

Figure 1 SEC chromatograms of EPS from activated sludge (AS-Liso) and anaerobic granular sludge (GS-Eer) with different detection mode (A) UV-Absorbance at 210 nm; (B) UV-Absorbance at 280 nm; (C) fluorescence (221 nm-350 nm) for Protein-like molecules and (D) fluorescence (345 nm-443 nm) for Humic like substances using a mobile phase (phosphate buffer (50 mM) with NaCl (150 mM) at pH 7.0 ± 0.1) at flowrate 0.5 mL.min^{-1} ((A), (B), (C), (D) Superdex HMW column, Amersham Biosciences).

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