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Water Research 39 (2005) 3044–3054

WATER RESEARCH

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Characterization of activated sludge exopolymers from various origins: A combined size-exclusion chromatography and infrared microscopy study

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Received 1 March 2005; received in revised form 3 May 2005; accepted 4 May 2005
Available online 5 July 2005

Abstract

High-pressure size-exclusion liquid chromatography and infrared microscopy were coupled to investigate the molecular weight and nature of extracellular polymeric substances (EPS) from various activated sludges. Six main families of compounds (proteins, polysaccharides, organic acids, lipids, mineral phases) were found either as a single molecule or as associations. The molecular weight of proteins varied from small (10 kDa) to large (600 kDa) sizes, while all polysaccharides were smaller than 1 kDa. Association of different molecules implied the presence of species large in size. The EPS chromatographic fingerprints of sludge from various origins remained stable in normal operating conditions, but were drastically modified during settling crises. In poor settling conditions, the EPS with smaller molecular sizes always prevailed and large polymers were underrepresented. The EPS identified in activated sludge were collected in a chemical database which provides the basis for comparison of municipal and industrial wastewater treatment plants (WWTP).

Keywords: Exopolymers; Proteins; Polysaccharides; Chromatography; Infrared micro-spectroscopy

1. Introduction

Extracellular polymeric substances (EPS), or exopolymers, are the construction materials of microbial aggregates in activated sludge flocs or biofilms. The term EPS includes different classes of macromolecules such as polysaccharides, proteins, nucleic acids (phospho)lipids and other polymeric compounds which fill and form the space between microbial cells. The EPS keep the organisms together in a three-dimensional gel-like hydrated matrix by weak physico-chemical interactions (electrostatic, hydrophobic, van der Waals and hydrogen interactions). The physicochemical properties of EPS are of interest for their contribution to the sludge flocculation and settling process. The extent of each bond contribution to the cumulative binding force depends strongly on the nature of EPS. Valuable information on the current knowledge about EPS can be found in a special issue (Flemming and Leis, 2001) and in some reviews (e.g. Wingender et al., 1999).

The optimization of the wastewater treatment process implies an understanding of interactions on a molecular basis. However, the heterogeneity and complexity of

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doi:10.1016/j.watres.2005.05.007

exopolymers represent an obstacle to such studies. Due to the lack of powerful investigation methods, these studies are often limited to the observation of the global impact of EPS or some of their components on wastewater treatment operations. The flocculating ability, the sludge settling and their relation to EPS properties are frequently studied but remain debated, and most often the results are controversial. Polysaccharides were suggested to play a major role in flocculation due to their negatively charged groups bridged by divalent cations (Flemming and Wingender, 2001; Körstgens et al., 2001; Sutherland, 1999; Higgins and Novak, 1997a, b). However, in many studies, proteins were found to be the main component within the sludge with a 4–5 protein/polysaccharide ratio; a relationship between protein content and floc formation was also observed (Liao et al., 2001; Fang and Jia, 1996; Urbain et al., 1993). The protein contribution to flocs binding strength is explained by hydrophobic interactions and polyvalent cation bridging, both enhancing the stability of the biopolymer network (Jorand et al., 1998, 1995).

The ability of EPS to interact with metallic ions is also advantageously involved in a biosorption of toxic metals such as Cd, Cu or Pb from wastewater in activated sludge treatment process. An interesting study on complexation abilities of extracted EPS was published by Guibaud et al. (2003), who used IR spectra to identify the presence of various complex-forming groups.

EPS play an important role in the structuration and water retention of biofilms and activated sludge. The dewatering and volume reduction of large amounts of sludge produced by wastewater treatment plants is an important problem. Advanced sludge treatment techniques (Neyens and Baeyens, 2003; Neyens et al., 2003, 2004), which degrade sludge EPS substances and thus reduce water retention, represent an economic challenge.

EPS complexity, both in nature and microenvironment, involves the use of separation techniques to extract the EPS from their matrix (valuable information can be found in e.g. Nielsen and Jahn, 1999; Frølund et al., 1996). The use of size-exclusion chromatography in the eighties revealed three or four families of macromolecules in extracted EPS samples in the $1–10^3$ kDa range (Forster, 1985; Horan and Eccles, 1986). In the last decade, the high-pressure size-exclusion chromatography (HPSEC) showed the presence of up to six or seven families of exopolymers (Frølund and Keiding, 1994; Frølund et al., 1996), but did not bring more information about the nature of macromolecules.

Recently, an original analytical approach (HPSEC combined with the Fourier transform infrared microspectroscopy (μFTIR)) was used to separate and identify complex EPS (Görner et al., 2003). This method has been used in the present study to inventory sludge exopolymers: (i) in one traditional wastewater treatment metropolitan plant over long periods (several months, even years) and thus in various seasonal conditions, (ii) in different plants treating domestic or (iii) industrial wastewater and (iv) in some cases, in crisis situations.

2. Materials and methods

2.1. Sample preparation and characterization

Activated sludge samples were collected from three metropolitan wastewater treatment plants (WWTP): Maxéville (300 000 inhabitant equivalents), Toul (24 500 inh. eqs.), Epinal (58300 inh. eqs.), referred to hereafter as WWTP M, WWTP T, WWTP E, respectively. Sludge samples were also taken from Boudonville sewer (Nancy) (sewer B) for comparison with municipal WWTP sludge, from one paper mill located in Etival (paper mill E) and one beer making plant in Champigneulles (beer plant C).

The grab samples were transported to the laboratory within 2 h and used for experiments. Different sludge characteristics were systematically measured by standard methods (Standard methods for the examination of water and wastewater, APHA-AWWA-WPCF, 1985): pH, conductivity, organic fraction (VS [g/L]), dry solid content (TS [g/L]), sludge volume index (SVI [mL/g]). The extraction procedure was adapted from Frølund et al. (1996): it uses a cation exchanger Dowex 50×8, 20–50 mesh H^+ form (Fluka, France) with a 4 h extraction time. Such a duration was selected from our kinetics studies as a good compromise between EPS conservation and extraction yield.

2.2. Size-exclusion chromatography

The separation of extracted EPS was carried out with a Hewlett-Packard 1100 Series chromatograph, equipped with a Zorbax Bio Series column (GF-250, 25 cm × 9.4 mm, Agilent Technologies, France) thermostated at 25 °C. The mobile phase (flowrate 0.5 mL/min) was made of 0.005 M NaCl and 0.005% NaN_3, pH 7.2 and ionic strength 0.01. This mobile phase is better than the phosphate phase used previously (Görner et al., 2003), as the obtained chromatographic fractions need not be dialyzed before μFTIR analysis. Indeed, the presence of NaCl precipitate does not interfere with organic matter recognition when using μFTIR.

Five protein standards of molecular mass 13 700, 45 000, 67 000, 200 000 and 670 000 Da (Ribonuclease, β-amylase, chicken albumin and bovine serum albumin), provided by Sigma Aldrich, were injected for column calibration, all within the linear range of retention times (30–24.5 min). The polysaccharide standards (180, 738, 5900, 11 800, 22 800, 47 300, 112 000, 212 000, 404 000