Colloids and Surfaces B: Biointerfaces 80 (2010) 161–168

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Effects of extraction procedures on metal binding properties of extracellular polymeric substances (EPS) from anaerobic granular sludges

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ARTICLE INFO

Article history:
Received 10 January 2010
Received in revised form 26 May 2010
Accepted 28 May 2010
Available online 4 June 2010

Keywords:
Extracellular polymeric substances
Extraction method
Binding properties
pK_a
Pb
Cd

ABSTRACT

The effects of the extraction procedure of extracellular polymeric substances (EPS) on their proton/metal binding properties were studied. Nine extraction procedures (one control, four physical and four chemical procedures) were applied to four types of anaerobic granular sludges. The binding capacities between the EPS and lead or cadmium were investigated at pH 7 by a polarographic method. The composition of the EPS extracts varied according to the extraction technique and the origin of the sludge. This induced differences in the pK_as and the binding sites density of the EPS extracts. The carry-over of the extractant in the samples strongly affects the properties of the EPS from chemical extraction protocols. Lead and cadmium seem to be bound differently with the EPS, a higher binding capacity was observed for Pb^{2+} than for Cd^{2+}.

1. Introduction

Anaerobic granular sludges are used to treat industrial or domestic high strength wastewater [1]. The granules display a high bacterial density, which allows the use of compact bioreactors. Granules are composed of bacteria embedded in an extracellular polymeric substances (EPS) matrix [2,3] composed of organic and mineral molecules. EPS originate from bacterial secretions or cell lysis products, but they can also adsorb organic or inorganic molecules from the wastewater [4]. EPS are composed of various biochemical molecules such as proteins, humic-like substances, polysaccharides, nucleic acids and lipids [4–6].

The EPS matrix strengthens the structure of the granules [7] but also has a protective role for the cells, adsorbing toxic pollutants present in wastewaters such as metallic elements (ME) [8]. These ME are mainly chemically bound to the bacterial cell membranes and the EPS of the biomass [8,9]. The ME biosorption is a sum of several physico-chemical processes: ion exchange reactions, complexation, adsorption and precipitation [10–12]. The sorption of metals by EPS is ascribed to their biochemical composition. The molecules composing the EPS contain ionisable functional groups such as carboxyl, hydroxyl or amino groups, which are involved in the metal binding. The abundance of these functional groups is related to the composition of the EPS. However, extraction procedures are known to affect the EPS composition [3–5], as previously shown for anaerobic granular sludges [6].

The main objective of this study was to gain a comprehensive view of the interactions between metal ions and EPS extracted from anaerobic granules. To test the potential effect of the EPS extraction method used, nine different EPS extraction methods were chosen to perform this study. First, the main functional groups present in the EPS samples were determined by infrared spectroscopy (IR). Then, the apparent acidic constants (pK_a) and the corresponding normalized concentrations of the binding sites were determined from acid–base titrations. Finally, the effect of EPS extraction procedures on the binding capacity (at pH 7.0) between the EPS and two ME (lead and cadmium) was investigated using a polarographic method.

2. Materials and methods

2.1. Sludge samples, EPS extraction and characterization

Four anaerobic granular sludges were sampled in bioreactors which treat various effluents such as wastewater of a paper-mill (Eerbeek), wastewater of a distillery (Nedalco) or vinasse of Brandy (Revico). The last sludge, Emmtec, was sampled from a lab scale bioreactor, which treats a SO_4^{2-}/ethanol synthetic effluent. Fur-

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doi:10.1016/j.colsurfb.2010.05.043

ther information regarding the respective sludge and wastewater characteristics are available in d'Abzac et al. [6].

According to the literature, there is no standard EPS extraction protocol. Nine extraction techniques, widely used in the literature, were therefore compared: a control method, four physical ones (sonication, cationic exchange resin (CER), CER + sonication and heating) and four chemical ones (formaldehyde + heating, formaldehyde + NaOH, ethylenediaminetetraacetic acid (EDTA) and ethanol). Note that Emmtec granular sludges was sampled from a lab scale bioreactor, so only a slight quantity of sludge was available and therefore only four EPS extraction methods were investigated. The extraction with centrifugation only was chosen as the control method because it is the least harsh extractive condition and the centrifugation step is common for all extraction techniques. The extraction protocols are described in detail in d'Abzac et al. [6].

The dry weight (DW) and volatile dry weight (VDW) of the EPS were, respectively, determined by the mass of samples after a drying at 105 °C for 24 h and the loss of mass after 2 h at 550 °C, respectively.

2.2. Determination of acidic constants of EPS samples

The EPS acid–base properties were determined from an acid–base titration. 50 mL of EPS solutions containing 0.25 g L^{-1} of EPS DW was placed in a 20 °C thermostated cell. Two separate titrations were performed with an automatic titrator (Metrohm 716 DMS Titrino) coupled to a Metrohm 727 Ti Stand, a Metrohm 722 stirrer and equipped with a pH electrode (Metrohm, pH 0–14/0; 80 °C; KCl 3 mol L^{-1}): one with HNO_3 0.1 mol L^{-1} (Normadose Prolabo) and the other one with NaOH 0.1 mol L^{-1} (Normadose Prolabo). Titrations were performed under nitrogen atmosphere (Linde, 5.0) in the cell after pH equilibrium was reached. The titrations were monitored and controlled by the Metrodata 716 DMS Titrino software program. The set parameters are the dynamic mode, a 2 mV min^{-1} signal drift and a 5 mL min^{-1} maximal flowrate.

The titration curves were modeled using Protofit (version 2.1) software [13] to calculate the pK_as values and the number of associated binding sites. For this calculation, the specific surface area was considered 1 m^2 g^{-1}. The non-electrostatic adsorption model and the Davis activity coefficient model were also chosen.

The acido-basic titrations of the EPS were performed on the 2 extracts of EPS obtained with the same method. The standard deviation for the pK_as and the number of sites varied less than 7%.

2.3. Polarographic investigation and metal complexation study

The binding capacity of EPS with Pb and Cd was studied through polarographic analyses. This method consists in determining the free metal content in the measuring cell after the addition of known volumes of metallic solution.

A Metrohm VA 663 static stand with three electrodes (a 3 M KCl/1 M KNO_3 reference electrode, a platinum counter electrode and a mercury drop working electrode) was used associated to a μAutolab Type III potentiostat/galvanostat. The polarographic study was monitored with the GPES (General Purpose for Electrochemical System) Manager Autolab software. The operating conditions were as follows: in the 20 °C thermostated (Lauda E100 thermostat) measuring cell, 1 mL of electrolyte (KNO_3, 1 M, Fluka, 99.995%), 10 mL of buffer (HEPES 0.5 M, pH 7, Sigma, 99.5%), 10 mL of ultra-pure water and 1 mL of EPS extract were mixed. Then, known volumes of Pb (10^{-3} M, Merck, $Pb(NO_3)_2$, 999 ± 2 mg L^{-1}, in HNO_3 0.5 M) or Cd (10^{-3} M, Merck, $Cd(NO_3)_2$, 1000 ± 2 mg L^{-1}, in HNO_3 0.5 M) solutions were added with a Metrohm 765 Dosimat automatic dispenser managed by the GPES software. The chosen measurement parameters are the static mode dropping polarography (SMDE) with a potential range scanned from −0.2 to −0.8 V, an intermediate mercury drop and a 1 s drop fall.

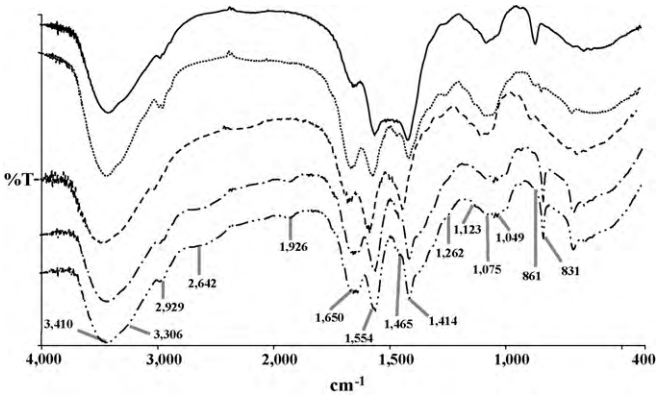

Fig. 1. IR spectra of EPS samples extracted with different physical EPS extraction procedures from Eerbeek sludge. (— centrifugation; . . . sonication; –··– CER and sonication; –·– CER; – – – heat.)

Before the measurement, the solution was stirred and subjected to a nitrogen flow during 15 min to reach the complexation equilibrium of the EPS with the metal investigated. The measurements after each addition of metal were performed in duplicate.

The theoretical study of the complexation equilibrium between EPS and metal associated with the law of mass action, assuming the formation of a 1:1 complex, can be described by:

$$M + L \leftrightarrows ML$$

with M as free metal, L as free ligand (EPS) and ML as metal–ligand complex.

Then the concentrations of EPS–Pb and EPS–Cd binding sites were determined using the Chau's graphic method [14]. The amount of EPS binding sites initially occupied was not taken into account for the determination of the total number of binding sites in EPS extracts due to the very low Cd and Pb content in extracted EPS (less than 1 per thousand). The extractions of EPS as well as the determinations of their abilities to bind Pb or Cd were performed twice, which allows to calculate an average value and a standard deviation of the number of sites present in each EPS extract.

2.4. Infrared spectrometry study

After freeze-drying, a mix of EPS (about 1 mg) and KBr (about 180 mg) was compressed. The formed pellets were analyzed using a spectrum 1000 IR spectrometer (PerkinElmer).

3. Results and discussion

3.1. Infrared investigation of EPS

Fig. 1 shows the infrared spectra of the EPS samples extracted with physical techniques from the Eerbeek sludge. The spectra for the other three sludges investigated are not presented but the trends and conclusions are similar: the same characteristic bands were observed on all the IR spectra, only the transmittance ratio of these bands varies from one sludge to another depending on the biochemical composition of the EPS extracts.

The various molecules composing EPS (proteins, polysaccharides, humic-like substances, nucleic acids, lipids... [4]) include different functional groups which can be identified by characteristic IR bands. The same characteristic bands are observed for IR spectra of samples extracted using the physical procedures investigated (Fig. 1). Some small differences concerning the transmittance ratios