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A high yield multi-method extraction protocol for protein quantification in activated sludge

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Abstract

A multi-method extraction protocol based on mechanical, ionic and hydrophobic methods was investigated on two types of activated sludge samples. Extraction methods were chosen with regards to optimal protein yield without cell disruption. Sonication, EDTA and Tween extraction methods were selected and combined. The total amount of protein released by the multi-method protocol sums up to 191 and 264 mg equiv. BSA/g VSS for the two different sludge samples. Protocol repetition on the same sample showed that protein yield after each successive protocol fitted an exponential curve model. The total amount of extractable proteins was evaluated by model predictions, 423 and 516 mg equiv. BSA/g VSS for the two sludge samples. The multi-method extraction protocol appears relevant for harvesting a representative quantity of proteins from the original sample (45–49%), moreover the multi-method criterion of the protocol also offers a heterogeneous pool of proteins. Thus, further qualitative studies may not be biased by the extraction protocol.
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1. Introduction

In biological wastewater treatment systems, bacteria aggregate together in order to form suspended floc structures or granules as well as attached biofilms. Several studies have shown that extracellular polymeric substances (EPS) take part in forming a gel-like matrix which acts as cement in bacterial aggregates. EPS are molecules produced by bacteria, brought by the incoming wastewater (Urbain et al., 1993) or are released from cells when lysis occurs. Humic acids (Frohlund et al., 1995), polysaccharides (Rideau and Morfaux, 1976), proteins (Dignac et al., 1998), nucleic acids (Jahn and Nielsen, 1997) and other non determined molecules (Wilén et al., 2003) have been reported as EPS compounds in the matrix. In spite of this wide range of macromolecules, recent studies have shown the relative importance of proteins in wastewater treatment systems from a quantitative aspect (Liao et al., 2001; Sponza, 2002), as well as from a functional point of view (Denecke, 2006). Proteins have been proven to increase biofilm adherence in bioreactors and cause fouling which has considerable consequences on water treatment by membrane filtration (Massé, 2004; Meng et al., 2006). Enzyme activity measurements showed that enzymes were integrated in the protein EPS pool (Frohlund et al., 1995) and that inherent proteases were the most active enzymes (Cadoret et al., 2002; Jung et al., 2002). Martinez et al. (2004) showed that protein content was linked to sludge processes, and that the types of proteins were rather linked to sludge settleability.

Total protein quantification and characterisation could therefore bring relevant information on bacterial aggregation processes as well as on treatment efficiency.

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Protein quantification is generally carried out by a prior extraction step. The most popular EPS extraction method is Cation Exchange Resin (CER), (Frohlund et al., 1995). Over many studies using this same method, proteins are showed as the predominant EPS in biofilm (Jahn and Nielsen, 1995) and in aerobic activated sludge extracts (Bura et al., 1998; Frohlund et al., 1995; Liu and Fang, 2002; Wilén et al., 2003). Sonication and formaldehyde extraction methods have also shown that proteins were the most represented EPS in activated sludge samples (Comte et al., 2006; Liu and Fang, 2002). However other extraction methods such as EDTA applied on the same sludge samples released more polysaccharides and humic substances than proteins (Denecke, 2006; Liu and Fang, 2002). The wide variety of extraction methods used in literature could partly explain the large variation of protein content reported in sludge, between 3.9 and 510 mg/g VSS (Liu and Fang, 2002; Massé, 2004; Wilén et al., 2003), without omitting other factors such as sludge origin and process designs which are also described as influencing EPS content (Comte et al., 2006; Sponza, 2002).

However, total protein quantification in sludge via extracts can be questionable since only one extraction method is carried out. Moreover, proteins are characterised by ionic, hydrophobic and neutral amino acids. Therefore, specific bonds such as electrostatic, hydrophobic and low energy hydrogen or Van der Waals interactions play a major role in linking proteins to the EPS matrix. Hence, the use of one type of extraction method is likely to promote the study of "one kind of protein" in quantification or characterisation studies. As shown by Dignac et al. (1998), extracellular proteins released by CER contained a majority of negatively charged amino-acids. General remarks in literature stipulate that quantification and characterisation of EPS compounds in sludge are often biased by extraction procedures which do not take into account the specificity of extraction methods towards certain types of EPS (Comte et al., 2007; Dignac et al., 1998). Thus, in order to assure a homogenous protein pool in extracts, it would be appropriate to apply three different extraction methods which aim on the three major types of bonds linking proteins to the EPS matrix, i.e. low energy, hydrophobic and ionic interactions. Protein quantification in sludge and sludge extracts is frequently carried out by modified Lowry method (Frohlund et al., 1995). However, studies have shown that bicinchoninic acid (BCA) is less sensitive to chemical extraction methods (i.e. EDTA), offers a better protein to protein response (Smith et al., 1985), as well as, a lower quantification limit (Ras et al., in press).

This present paper offers a multi-method extraction protocol based on three types of extraction methods (mechanical, ionic and hydrophobic) in order to obtain a diversified protein pool which can stand for the studied sludge.

Several extraction methods were chosen to be investigated according to their frequency in literature and care towards cell disruption and protein denaturation. This includes mechanical and ionic extraction methods such as sonication (Azeredo et al., 1998; Comte et al., 2006; Dey et al., 2006), cation exchange resin (Cadoret et al., 2002; Comte et al., 2006; Park and Novak, 2007; Sesay et al., 2006), and EDTA (Comte et al., 2006; Liu and Fang, 2002; Sheng et al., 2006). EPS extraction methods which aim hydrophobic interactions in sludge are randomly found in literature. However, among the several non ionic surfactants employed in previous studies, Tween and ethanol have been investigated as hydrophobic extraction methods.

Each method was controlled for their possible effect on cell disruption and protein denaturation, as well as, for their interference towards the protein quantification method. The most efficient methods were selected and applied in series according to an original three step extraction strategy. This study will also give more information on the total amount of extractable proteins which can be released from activated sludge.

2. Methods

2.1. Activated sludge samples: origin and handling

The extraction methods were undertaken on sludge samples from two different pilots. Organic supply and sludge characteristics are described in Table 1.

Samples were collected the previous day, stored at 4 °C and diluted with a Phosphate Buffer Saline (PBS, pH 7) to 5 g VSS/L of sludge before each extraction. Total solids (TS) were measured by desiccating at 105 °C and volatile suspended solids (VSS) were measured by calcinate at 510 °C.

2.2. Extraction instruments and chemicals

Extraction tests were undertaken on 50 mL sludge samples. Stirring intensities were all performed at 500 rpm.

2.2.1. Mechanical extractions

Ultrasounds were carried out with a vibra cell sonication probe from Bioblock (2.5 mm × 49 mm cylinder).

Table 1
Pilot and sludge characteristics

		Pilot 1	Pilot 2
Charge	g DCO/L/d	0.2	0.06
Treated effluent	Type	Domestic wastewater	Synthetic (propionate/acetate/starch/ethanol)
Sludge residential time (SRT)	Days	8	8
Sludge concentration	g TS/L	1.5	1.5
Dissolved oxygène	mg/L	3	3
Oxygenation time	min	40	40
Anoxic time	min	40	40
Volatile suspended solids/total solids	g VSS/g TS	0.8–0.85	0.8–0.85
Temperature (°C)	20	20	20

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