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Application of enzymes, sodium tripolyphosphate and cation exchange resin for the release of extracellular polymeric substances from sewage sludge Characterization of the extracted polysaccharides/glycoconjugates by a panel of lectins

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Abstract

The study describes extraction of extracellular polymeric substances (EPS) from sewage sludge by applying enzymes and enzymes combined with sodium tripolyphosphate (STPP). Additionally, a systematic study of two non-enzymatic extraction agents is described. The assessment of the released products is made by colorimetrical methods and polysaccharides/glycoconjugates identification by the interaction with four immobilized lectins. Bio-sludge from Helsingborg (Sweden) and Damhusåen (Denmark) were used as two case studies for testing enzymatic extractability and thereby to make useful prediction of sludge bio-digestibility. From Helsingborg sludge the enzymes extracted about 40% more of EPS than from Damhusåen. The polysaccharides/glycoconjugates in both sludges maintained the same level, and showed substantial different interaction motifs with lectins panel. Damhusåen enzymatic extracted EPS had an enhanced amount of suspended material that was post-hydrolysed by the use of polygalacturonase and lysozyme resulting in pectin like polymers and petiptidoglycans. Petiptidoglycan is a marker from bacterial cell debris. STPP and cation exchange resin (CER) released different quantities of EPS. The CER released polysaccharides/glycoconjugates had higher molecular weight and stronger affinity towards Concanavalin A than the one released by the action of STPP. Independent of the extraction conditions, STPP released elevated amounts of polyvalent cations and humic substances in contrast to the very low amounts of released by CER.

Keywords: Bio-sludge; Cation exchange resin; Enzymes; Extracellular polymeric substances; Lectin; Sodium tripolyphosphate

1. Introduction

The activated sludge process is commonly used in wastewater treatment. This process is based on the aerobic digestion of organic matter by floc-forming microorganisms. Polymeric network of activated sludge flocs is composed of extracellular polymeric substances (EPS) (Liu and Fang, 2003). The EPS originate from bacteria active secretion (Prescott et al., 2002) and organic or inorganic debris present in sewage sludge itself (Tchobanoglous et al., 2003). EPS are composed of a variety of organic substances: carbohydrates, proteins, humic substances, uronic acids, lipid compounds and deoxyribonucleic acids. EPS act together with multivalent ions to aid the formation and settling of sludge flocs in both aerobic and anaerobic treatment systems. On the other hand, an excess of EPS may hinder dewatering of sludge, bio-flocculation and settling of sludge (Forster and Lewin, 1972; Liu and Fang, 2003; Pere et al., 1993; Urbain et al., 1993)

There have been described various EPS extraction methods based on physical, chemical, and biological treatment (Comte et al., 2006; Liu and Fang, 2003; Van Lier et al., 2001). The amount of extracted EPS from the same batch of sludge varies widely as a function of extraction conditions (Liu and Fang, 2003). To remove polymers from the sludge floc structure, chemical

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extraction techniques such as acids and bases (Brown and Lester, 1980; Rudd et al., 1983), chelating agents, e.g., EDTA (Brown and Lester, 1980; Eriksson and Alm, 1991) and EGTA (Bruus et al., 1992; Sanin and Vesilind, 2000) or cation exchange resins (CER) (Durmaz and Sanin, 2001; Froelund et al., 1996; Liao et al., 2001) were used.

A few papers deal with enzymatic hydrolysis of sludge (Cadoret et al., 2002; Jansen et al., 2004; Jung et al., 2002; Kim et al., 2002; Mayhew et al., 2002; Sesay et al., 2006; Wawrzynczyk et al., 2003; Whiteley et al., 2002, 2003). Our recent findings showed that enzymes are useful both for releasing EPS and in the identification of polysaccharides and glycoconjugates together with lectins panel (Dey et al., 2006). Hydrolytic enzymes break down polymeric substances like proteins, polysaccharides, lipids, and additionally can cause a release of macromolecules, e.g., humic substances, that are non-specifically bound to the mentioned substrates (Ji and Brune, 2005).

Lectins are sugar-binding proteins that agglutinate cells and/or precipitate complex carbohydrates (Cummings, 1994). Plant lectins are extensively used in purification, detection and structural characterization of glycoconjugates, investigation of cell-surface architecture, blood typing, identification and differentiation of various prokaryotic and eukaryotic cells and as epidemiological or taxonomic markers (Lis and Sharon, 1998).

Surface plasmon resonance (SPR)-Biacore based detection has been previously used to study the interaction between the carbohydrates and lectins (Sota et al., 2003). Our previous findings showed a potential of SPR based detection for characterization of organic material extracted from sludges (Dey et al., 2006).

In the work described in this paper we applied enzymes, sodium tripolyphosphate and cation exchange resin for the release of EPS from sewage sludge. Enzymatic treatment had two purposes: (i) to release EPS and (ii) to characterize the released material by specific cleavage. The extracted polysaccharides and glycoconjugates were characterized by a panel of lectins immobilized on SPR sensor surface.

2. Materials and methods

2.1. Sludge handling

Surplus biological sludge (bio-sludge) was obtained from three different municipal wastewater treatment plants (WWTP): Lund (Sweden), Helsingborg (Sweden) and Damhusåen (Denmark). The sludge was pretreated as described earlier (Wawrzynczyk et al., 2003). Freshly collected sludge was settled for 4 h at 4 °C, further concentrated by centrifugation (3500 g, 10 min, 4 °C) and then stored at 4 °C overnight before use. The characteristics of the sludges used were: soluble COD $200 \pm 50\,mg\,l^{-1}$, volatile solids (VS) 13.6–14.4 $g\,l^{-1}$, and total solid (TS) always adjusted to $20\,g\,l^{-1}$.

2.2. Analytical methods

All reagents used were of analytical purity. If not stated in the text, all determinations were done in the liquid phase. COD was determined using cell kits from Merck. TS and VS were determined in accordance with standards methods (APHA, 1995). The carbohydrate concentration was determined using the anthrone-sulfuric acid method (Gaudy, 1962). A method of standard additions and a calibration curve made with glucose were used to calculate the analyte concentrations. The quantification limit was of $10\,\mu g\,ml^{-1}$.

The Lowry method was applied for protein determination, according to Froelund et al. (1996). The protein concentration was determined from a calibration curve made from Bovine Serum Albumin (BSA, Sigma) and the limit of quantification was of $10\,\mu g\,ml^{-1}$. The modified Lowry method was used for determination of humic compounds according to Froelund et al. (1995). The concentration of analyte was calculated from the calibration curve made for standard humic compounds (Calbiochem). The quantification limit was of $20\,\mu g\,ml^{-1}$.

The Inductive Coupled Plasma method (ICP) (4300 V Perkin-Elmer) was used to detect metal ions concentration in the liquids. Analysis was performed at the Kemira Kemi AB, Kemiteknik, Analytical Service, Helsingborg (Sweden).

The total measured EPS (ΣEPS) represents mathematical sum of all measured compounds (proteins, humic substances and carbohydrates) for each particular sludge and treatment conditions.

2.3. Extraction methods

(i) *Enzymatic sludge pre-treatment and post-treatment* were performed as described previously (Dey et al., 2006). Enzymes: protease, lipase, cellulase, alpha-amylase, dextranase, endo-xylanase, and polygalactouronase were gifts from Novozyme A/S, Denmark and were of technical grade. Lysozyme from chicken egg was purchased from Sigma, USA. The specificities of the used enzymes were described previously (Dey et al., 2006).

(ii) *Extraction with sodium tripolyphosphate (STPP)*. Pulverized STPP (Sigma, USA) was added directly to pre-concentrated sludge (TS 2%), to a final concentration of 50 mM. The extractions with STPP were carried out at constant pH 7 or 9. The pH was adjusted to 7.0 using 1 M HCl.

(iii) *Extraction with cation exchange resin (CER)*. For extraction with CER, Amberlite resin IRC-50(H) (50 × 8), 20–50 mesh, an analytical grade, weak cation-exchanger (BDH Chemical Ltd., Poole, England) was used. The CER was used at constant pH of 4 and 7. After addition of CER to the sludge the pH of sludge mixture dropped to 4, therefore sludge pH was adjusted to 7 with 1 M NaOH. The amount of CER used for the extraction of EPS from bio-sludge was 60–80 $g\,g^{-1}$ of CER per VS. The resin was washed before extraction with 1 mM phosphate buffer (pH 7.0) for 1 h at 4 °C.

Extractions were performed at two temperatures: 4 °C and 45 °C, and sludges were mixed with a propeller stirrer at 200 rpm for 4 h. The EPS was harvested by an initial centrifugation of sludge and obtained liquid phase was re-centrifuged (7000 × *g*,