A NEW METHOD FOR EXTRACTION OF EXOPOLYMERS FROM ACTIVATED SLUDGES

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ABSTRACT

The exopolymeric matrix that surrounds the biomass greatly contributes to the efficiency of activated sludge systems. To study the composition of this matrix a preliminary extraction method is required. In this work three extraction methods (vapor, sonication and combined treatment by sonication and Dowex resin) were used and compared with a new method which uses glutaraldehyde. The extraction effectiveness was estimated by measuring the total protein, the total organic carbon in the extracted solutions and by determining the monosaccharide constituents of the extracted polysaccharides. The proposed new method proved to be the most suitable because it extracted a great quantity of organic matter without disrupting the cells. © 1998 IAWQ Published by Elsevier Science Ltd

KEYWORDS

Activated sludge; biopolymer extraction; exopolymers; glutaraldehyde; ion exchange; polysaccharides; proteins; sonication; vapor.

INTRODUCTION

Activated sludge flocs are complex systems composed of microbial cells embedded in a polymeric matrix. The exopolymer matrix has been defined as "materials which can be removed from microorganisms without disrupting the cells and without which the microorganism is still viable" (Gehr and Henry, 1983). Numerous research projects have been devoted to the extracellular matrices to pinpoint their role in activated sludge flocculation (Vallom and McLoughlin, 1984; Goodwin and Forster, 1985; Horan and Eccles, 1986). The extracellular matrix of the biofilm is often termed "biopolymers" or "polysaccharides". In fact, although polysaccharides predominate and represent up to 65% of extracellular materials (Horan and Eccles, 1986), other substances are also present such as proteins, nucleic acids and lipids (Goodwin and Forster, 1985). Some exopolymers contain glycoproteins, glucosides or glucon phosphates (Ford et al., 1991).

Almost all the information concerning the chemical composition of the exopolysaccharides has been obtained through analyses made directly on activated sludge samples (Lazarova and Manem, 1995). Some extraction steps are available to study the composition of this complex exopolymer matrix. These include ion-exchange and thin layer chromatography (Goodwin and Forster, 1985; Horan and Eccles, 1986), NaOH, EDTA or
vapor extraction (Frølund et al., 1994; Brown and Lester, 1980). Most of these methods have a very low efficiency in terms of selective exopolymer extraction and promote cellular lysis or intracellular material loss which can distort the results.

The aim of this work is to select an exopolymer extraction method with a high efficiency and a minimum amount of cell lysis. This goal is achieved by comparing the effectiveness of different methods described in the literature with that of a proposed new method.

MATERIAL AND METHODS

Activated sludge samples

The extraction methods were tested on activated sludge obtained from the aeration basin of a wastewater treatment plant in Plaisir (France).

Extraction methods

Extraction with glutaraldehyde. The activated sludge was harvested by centrifugation (9000 g, 20 min) and washed with water prior to extraction. The pellet was divided into four equal parts of 3 g each that corresponded to 0.1807 g of sludge dry weight (dw). Two parts were resuspended in 15 ml and 30 ml of 3% (v/v) glutaraldehyde giving final concentrations of 1.2% and 0.6% (dw/v) of sludge respectively. The other portions were resuspended in 15 ml and 30 ml of 10% (v/v) glutaraldehyde.

The sludge suspension was incubated overnight at 4°C under slow agitation (50 rpm). Each sample was centrifuged at 9000 g for 20 min.

Extraction with vapor. After being harvested and washed, 3 g activated sludge was treated by the procedure described by Brown and Lester (1980).

Extraction by sonication and Dowex resin. The activated sludge was centrifuged for 15 min at 3000 g at 4°C and the pellet was resuspended in a phosphate buffer of the same volume as the initial sludge sample. This suspension (100 meq) was sonicated for 1 min with a 13 mm probe (300 W sonicator, Bioblock), immersed 25 mm in the liquid, using a power output of 37 W. The tubes containing the samples were kept in crushed ice during sonication. The extracellular polymers were obtained in the aqueous solution after centrifugation of the sonicated samples at 9000 g for 20 min. When using the Dowex resin, 30 g of resin (50X8, Na+ form, 20-50 mesh, Aldrich-Fluka 44445) were added to the 100 ml sonicated fractions. The suspensions were agitated at room temperature for 1 h and centrifuged at 9000 g for 20 min.

The resulting supernatants of all the extraction methods were dialysed using a membrane of 14000 MWCO (Medicell, dialysis tubing-visking) against ultra-pure water for 2 days at 4°C.

Analytical methods

Total organic carbon TOC. Samples were first acidified with phosphoric acid (final pH 2-3) and the inorganic carbon fraction was removed by aeration. The total organic carbon was measured on a Beckman analyzer (Tocamaster™, Model 915B).

Total protein and sugars. Total protein was determined by the Lowry modified method, using the protein assay kit SIGMA P5656 with a standard of BSA (bovine serum albumin). Sugars were analyzed after alditol acetates derivatization (Fox et al., 1989) by GC chromatographic analysis on a fused-silica capillary column (SP 2380 Supelco) with an FID detector.
RESULTS AND DISCUSSION

The TOC and the total protein extracted by the three extraction methods tested are presented in Fig. 1. The results obtained were compared with the total protein content, measured in a sludge sample after total cell disruption by 10 min of sonication, with a mean value of 43.5% (w/dw). After 20 min of vapor extraction the same quantity of protein was obtained. Increasing the duration of the vapor extraction produced more proteins suggesting that more intracellular material had been released. All other extraction methods tested detected a smaller amount of protein. Glutaraldehyde extraction yielded the smallest quantity of protein, (11% w/dw).

Although the duration of the sonication was optimized to produce a minimum of cellular lysis (Pierzo et al., 1994), the high quantity of proteins extracted could not only be the result of the residual cellular lysis above; it might also be due to materials extracted from the periplasmic space (Neidhardt et al., 1990). It is worth noticing that sonication followed by Dowex resin extracted less protein than sonication alone. The Dowex resin retains positively charged compounds, so it is possible that some proteins and amino-sugars could be captured by this resin due to the low pH of the solution.

![Figure 1. Total organic carbon and total protein extracted by sonication, vapor and glutaraldehyde.](image)

The TOC analysis quantifies all the organic matter extracted, be they proteins or polysaccharides. The ratio proteins/TOC was greater for sonication than for glutaraldehyde extraction, indicating that more polysaccharides were obtained with the glutaraldehyde extraction. The GC analysis of the extracted samples was made to verify this statement.

The results obtained on the composition of polysaccharides (Fig. 2) agree well with the data reported by Horan and Eccles (1986) and show the predominance of a limited number of monomers such as glucose, mannose and fucose. Vapor extraction extracted the greatest quantity of sugars. It is also clear that ribose is present in large amounts when compared to its value with the other methods. This monomer is a structural sugar, so it can be assumed that the large quantity of ribose is due to the cellular lysis caused by heating.

The methods that extracted the smallest quantity of ribose were glutaraldehyde and Dowex resin. Part of the molecules released by sonication were retained in the resin, resulting in a smaller quantity of ribose, which was the same quantity extracted by glutaraldehyde. Galactose and mannose are also constituents of lipopolysaccharides (Neidhardt et al., 1990) and this may explain why these monomers appear in higher quantities when vapor extraction and sonication were used.

The ratio TOC/protein was higher for glutaraldehyde, although the quantity of monomers was smaller compared to sonication. It is important to stress that only monosaccharides were in the extracted solutions. Other organic materials should also be present, such as glycoproteins, nucleic acids and fatty acids.
Glutaraldehyde extraction was optimized by testing two concentrations and two volumes of glutaraldehyde. When using equal volumes of glutaraldehyde solutions at 3% and 10% the quantity extracted was almost the same. This means that glutaraldehyde concentration has a minor effect on extraction yield. When glutaraldehyde is contacted with the polymeric matrix it appears to solubilize its constituents, until saturation is reached. Therefore, as more glutaraldehyde is added, a larger quantity of organic matter is extracted.

Figure 2. Monomer composition of the sludge polysaccharides extracted by sonication, vapor and glutaraldehyde.

CONCLUSIONS

Vapor extraction was not suitable because a great amount of intracellular material was extracted. Sonication promoted the excretion of large quantities of proteins indicating cellular lysis or breakage of the cell membrane. Glutaraldehyde was the most suitable method for biopolymer extraction, because it produced a high TOC/protein ratio and had no disruptive effect on the biomass. The yield of biopolymer extracted increased with the volume of glutaraldehyde added to the sludges.

REFERENCES


