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 (vapour, sonication and combined treatment by sonication and Dowex resin) were used and compared with a new method which uses glutaraldehyde. The extraction's effectiveness was estimated by measuring the total protein content, the total organic carbon in the extracted solutions and by determining the monosaccharide constituents of the polysaccharides extracted. The new method proposed proved to be the most suitable one, as it extracts a great quantity of organic matter without disrupting the cells.

KEYWORDS

Activated sludge, biopolymer extraction, exopolymers, glutaraldehyde, ion exchange, polysaccharides, proteins, sonication, vapour.

INTRODUCTION

Activated sludge flocs are complex systems composed of microbial cells embedded in a polymeric matrix. The matrix of exopolymers 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 glucophosphates (Ford et al. 1991).

Almost all the information concerning the chemical composition of the exopolysacharides has been obtained through the analysis 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 including ion-exchange and thin layer chromatography (Goodwin and Forster 1985; Horan and Eccles 1986), NaOH, EDTA or vapour 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 characterized by high efficiency and minimal effect on cell lysis. This goal is achieved by comparing the effectiveness of different methods described in the literature with a proposed new method.

MATERIAL AND METHODS

Activated sludge samples

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

Extraction methods

Extraction with glutaraldehyde. The activated sludge was harvested by centrifugation (9000g, 20min) and was washed with water prior to the extraction. The pellet was divided into four equal parts of 3g each that corresponds to 0.1807g of sludge dry weight (dw). Two parts were ressuspended on 15ml and 30ml of 3% (v/v) glutaraldehyde giving a final concentration of 1.2% and 0.6% (dw/v) of sludge respectively. The other portions were ressuspended on 15ml and 30ml of 10% (v/v) glutaraldehyde.
The sludge suspension was incubated overnight at 4°C under slow agitation (50 rpm). Each sample was centrifuged at 9000g during 20 min.

Extraction with vapour. After being harvested and washed, 3g of the activated sludge was submitted to the procedure described by Brown and Lester, 1980.

Extraction by sonication and Dowex resin. The activated sludge was centrifuged during 15 min at 3000 g at 4°C and the pellet was ressuspended on a phosphate buffer to the initial volume of the sludge. 100 ml of the suspension were 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 9000g during 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 during 1 hour and centrifuged at 9000 g during 20 min.

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

Analytical methods

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

Total proteins and sugars. The total proteins were 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 derivatisation (Fox et al. 1989) by GC chromatographic analysis on a fused-silica capillary column (SP 2380 Supelco) with a FID detector.

RESULTS AND DISCUSSION

The total organic carbon (TOC) and the total protein extracted by the three extraction methods tested are presented in Figure 1. The results obtained were compared with the total protein content, measured in a sludge sample after total cell disruption by 10 minutes of sonication, with a mean value of 43.5% (W/DW). After 20 minutes of vapour extraction the same quantity of protein was obtained. Increasing the duration of the vapour extraction, a greater quantity of proteins is measured, suggesting that more intracellular material

is released. All the other extraction methods tested detected a smaller amount of protein. Glutaraldehyde extraction is the method that extracts the smaller quantity of protein, (11% W/DW).

Although the duration of the sonication was optimized to reach 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, but may also be due to materials extracted from the periplasmic space (Neidhardt et al. 1990). It is worth noticing that sonication followed by Dowex resin extracts 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 solutions.

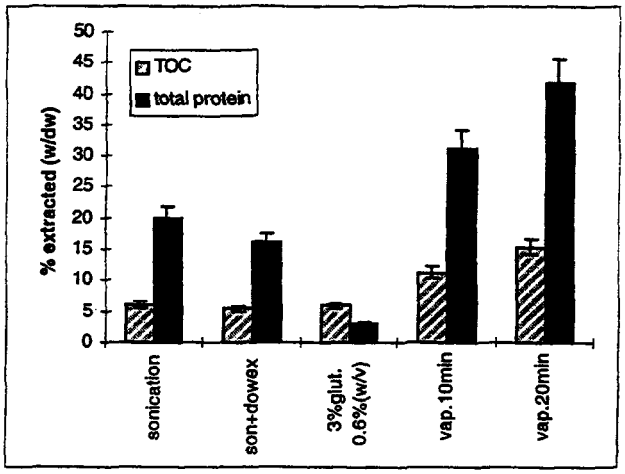

Fig 1 Total organic carbon and total protein extracted by sonication, vapour and glutaraldehyde

The TOC analysis quantified all the organic mater extracted, proteins and polysaccharides likewise. The ratio proteins/TOC is greater for sonication than for glutaraldehyde extraction, indicating that more polysaccharides are 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 Ecles (1986) and show the predominance of a limited number of monomers such as glucose, mannose and fucose. Vapour extraction is the method that extracts the greatest quantity of sugars. It is also clear that ribose is present in large amounts, when compared with the other methods. This monomer is a structural sugar, so it can be assumed that the great quantity of ribose is due to the cellular lysis caused by heating.

The methods that extract the smallest quantity of ribose are 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 vapour extraction and sonication are used.

The ratio TOC/protein was higher for glutaraldehyde, although the quantity of monomers was smaller compared with sonication. It is important to stress that only monosaccharides were searched for in the extracted solutions. Other organic materials are also expected to be present, such as glicoproteins, nucleic acids and fatty acids, among others.

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 is almost the

same. This means that glutaraldehyde concentration has a minor effect on the yield of extraction. When glutaraldehyde is put into contact with the polymeric matrix it appears to solubilise its constituents, until saturation is reached. Therefore, as more glutaraldehyde is added, a larger quantity of organic matter is extracted.

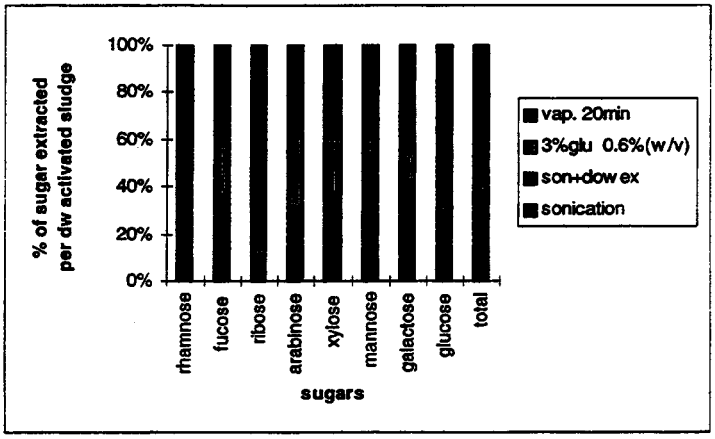

Fig. 2 Monomolar composition of the sludge polysaccharides extracted by sonication, vapour and glutaraldehyde.

CONCLUSIONS

Vapour extraction did not meet the requirements because a great amount of intracellular material was extracted. The sonication promoted the excretion of a great quantity of proteins indicating a possible cellular lysis or breakage of the cell membrane. Glutaraldehyde proved to be the most suitable method for biopolymer extraction, as it presented a high ratio TOC/protein and had no disruptive effect on the biomass. The yield of biopolymer extracted increased with the volume of glutaraldehyde added to the sludges.

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