THE ROLE OF EXTRACELLULAR EXOPOLYMERS IN THE REMOVAL OF PHOSPHORUS FROM ACTIVATED SLUDGE

T. E. CLOETE and D. J. OOSTHUIZEN

INTRODUCTION

Removal of phosphorus from domestic and industrial wastewater is central in curbing the water pollution problem of eutrophication, a process resulting in excessive growth of algae and other photosynthesizing water plants (Cloete and Bosch, 1994). For this reason, many countries impose limits on the phosphate content of effluents to rivers and dams. Removal of phosphorus from wastewater is only possible by incorporation of phosphate into a solid phase, which may subsequently be separated from the water phase (Erasmus, 1997). Such processes include chemical precipitation and biological phosphate removal.

In terms of biological wastewater treatment, the activated sludge process is probably today's most important biotechnological process. Although a considerable amount of work has been done on system design and process engineering, most systems designed for enhanced biological phosphorus removal (EBPR) routinely fail, necessitating chemical precipitation to meet effluent standards. Studies on the microbial ecology of activated sludge in order to optimize the process have received much attention. Classic culture-dependent, as well as molecular techniques, like fluorescent antibody and hybridization approaches and polyacrylamide gel electrophoresis (PAGE) have contributed to a better, but as yet incomplete understanding of the EBPR process. This is attributed to the intrinsic limitations of techniques used to study microbial population dynamics in natural habitats. Hence, microbial diversity and, more importantly, the function of populations in a specific community have not been elucidated. To date, EBPR has not been attributed as a function of a specific microbial population in activated sludge. This might be because the utilization of phosphate is common to all bacteria, both as energy source and a building block of genetic material, making it difficult to correlate EBPR with only one group of microbes (Mino et al., 1998). Furthermore, although the macro-environmental conditions of activated sludge have been well described, very little is known about the micro-environment in activated sludge flocs, including diffusion gradients and the role of EPS in EBPR.

A large part of the floc structure in activated sludge is composed of EPS and attachment of microbial cells in a floc structure is thought to be due to the presence of these exopolymers. These polymers are composed of sugars, amino acids and uronic acids (Bitton, 1994). Adsorptive properties of exopolymers have been well documented especially in terms of biosorption of pollutants and toxics (Beech and Cheung, 1995; Loaëc et al., 1997). However, the role of EPS in biological phosphorus removal has not been well studied. In previous studies, Buchan (1980) used EDS to determine the location of phosphorus volutin granules in activated sludge, without considering the phosphorus content of EPS.

EDS entails the bombardment of a sample with electrons, producing, along with various secondary signals, X-rays. The release of X-rays is produced by ionization of atoms in the sample. This ionization creates a vacancy in one of the energy levels of the atoms, which is almost immediately filled by an electron from a higher energy level. As these electrons transfer to lower energy levels, the excess energy is emitted as X-rays (Buchan, 1983). Each element in the periodic table contains electrons in orbits with particular discrete energy levels, and it is on the basis of the differences between these specific energies that each element can be identified (Buchan, 1983). The spectrometer plots energies of different X-rays against counts of each specific energy, giving both qualitative and quantitative information about the elemental composition of a sample. EDS was, therefore, chosen as a technique to study the role that EPS plays in phosphate removal from wastewater in the activated sludge process.

MATERIALS AND METHODS

Sampling

Two EBPR plants situated in Pretoria, South Africa and functioning exclusively biological (i.e. without chemical addition to attain phosphate limits) were sampled. Both plants had the same configuration (three-stage Bardenpho) and sludge age (12 days). To standardize the experiment, 500 ml of sludge at the end of the aerobic and anaerobic zones were drawn into sterile Schott bottles and transported on ice to the laboratory for processing. *o*-phosphate analyses were done on the influent wastewater, as well as sludge at the end of the aerobic zone directly before secondary settling. These analyses were done immediately after sampling for the Daspoort EBPR and 45 min after sampling for the Rooiwal EBPR. The analyses were done using a SQ118 spectrophotometer (Merck) and the relevant P(VM) test kit after filtration of 10ml of sample through Whatman no. 1 filter paper.

Sample preparation

To remove loosely bound phosphate from the EPS, 1ml of sludge from every sample was transferred to sterile 1.5 ml. Eppendorf tubes and washed three times with sterile double distilled water by centrifugation at 10,000 rpm for 5 min. The biomass pellets were finally resuspended in 1ml of sterile double distilled water. The samples were then diluted 1: 10 in sterile double distilled water and 10ml spotted on high purity carbon stubs. These were left to air-dry at 378C before being coated under vacuum with a 25nm layer of high purity carbon. Two controls consisting of only a carbon stub without sample and a carbon stub containing only 10 ml of sterile double distilled water were included to check the purities of both the carbon and double distilled water.

EDS Analysis

Samples were analysed by means of EDS. The scanning electron microscopy (SEM) used was a Jeol model JSM-5800LV using a backscatter detector for compositional contrast. Samples were analysed by a pre-standardized Noran Voyager system at 15keV for a lifetime of 100s. Ten analyses of each sample were done. Digital images were captured by means of an Orion frame-grabber.

RESULTS AND DISCUSSION

At the time of sampling, both the EBPR plants produced effluents which conformed to the South African 1 mgl^{-1} standard for *o*-phosphate discharged to water sources.

There are many problems associated with EDS of biological samples as normal preparation techniques for electron microscopy can displace, transform and dissolve many elements within biological samples, and therefore, not representative of the *in vivo* situation (Buchan, 1980). In this study, we avoided normal SEM preparation to minimize the above mentioned problems.

SEM showed that the Daspoort and Rooiwal EBPR sludges consisted not only of free bacteria and filamentous organisms, but mostly of bacteria in clusters encapsulated by EPS (Figs 1 and 2).

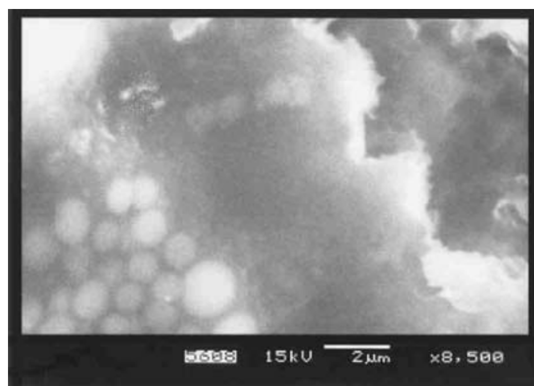

Fig. 1. Backscatter electron micrograph indicating the spatial distribution of cell clusters and EPS in a Daspoort EBPR sludge sample. Note the compositional contrast.

Table 1. Summary of results for EDS analysis of cell cluster and EPS phosphorus content of different activated sludge plants and different zones within a particular plant (Daspoort). Percentages represent averages of phosphorus content of ten fields analysed per sample

Description	Range %	Average %	SD[a](%)
Daspoort			
Cell clusters (anaerobic zone)	55-60	58	2
Cell clusters (aerobic zone)	51-59	57	2
EPS (anaerobic zone)	23-37	30	8
EPS (aerobic zone)	19-33	27	4
Rooiwal			
Cell clusters (anaerobic zone)	56-64	59	3
EPS (anaerobic zone)	16-35	29	8

On average, cell clusters with associated EPS from the two EBPR sludges contained between 57% and 59% phosphorus (Table 1, Figs 1–3), while EPS alone contained between 27% and 29% phosphorus (Table 1, Figs 1, 2 and 4). These values showed little variation between the two plants sampled and different zones within a particular plant (Daspoort}Table 1).

As counter-ions to phosphorus, magnesium and potassium were dominant in cell clusters, while sulphur and silicon were also found in the EPS (Table 2).

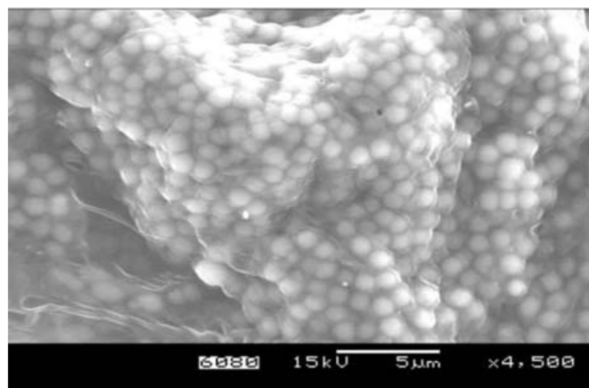

Fig. 2. Backscatter electron micrograph indicating the spatial distribution of cell clusters and EPS in a Rooiwal EBPR sludge sample. Note the compositional contrast.

For the Daspoort EBPR sludge, it was easy to distinguish the bacterial cells from the EPS making EDS analysis easy (Fig. 1). However, due to the very close encapsulation of cells by the EPS in the Rooiwal EBPR sludge (Fig. 2), EDS analysis was more difficult. Areas of clear EPS (without embedded cells) large enough to prevent overlap of X-rays emitted from both cells and EPS during analysis were identified with difficulty.

Further attempts to optimize the EDS analysis should be investigated and include transmission electron microscopy (TEM) in combination with EDS to avoid large excitation volumes experienced in this report. Preparation of samples should include rapid freezing or cryo-sectioning to avoid displacement and/or migration of ions inside the samples. Also, attempts should be made to separate EPS from cells to avoid regions of overlap between EPS and cells and to facilitate bulk analysis on EPS.

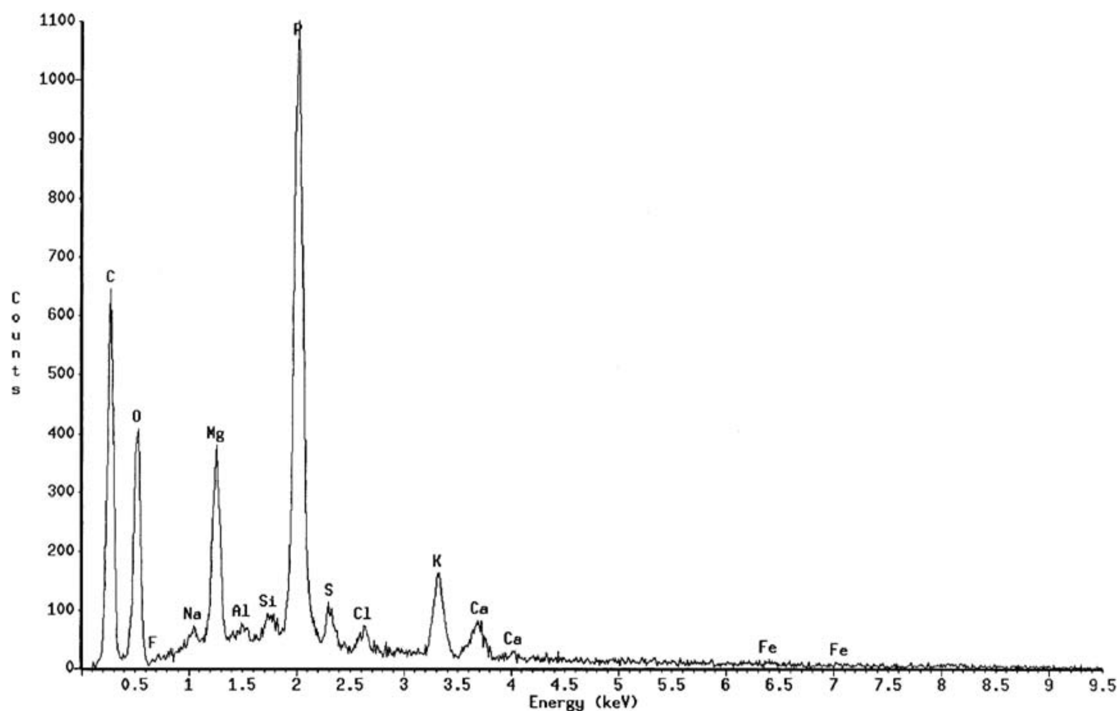

Fig. 3. Typical analytical X-ray spectrum of Daspoort EBPR anaerobic sludge cell clusters.

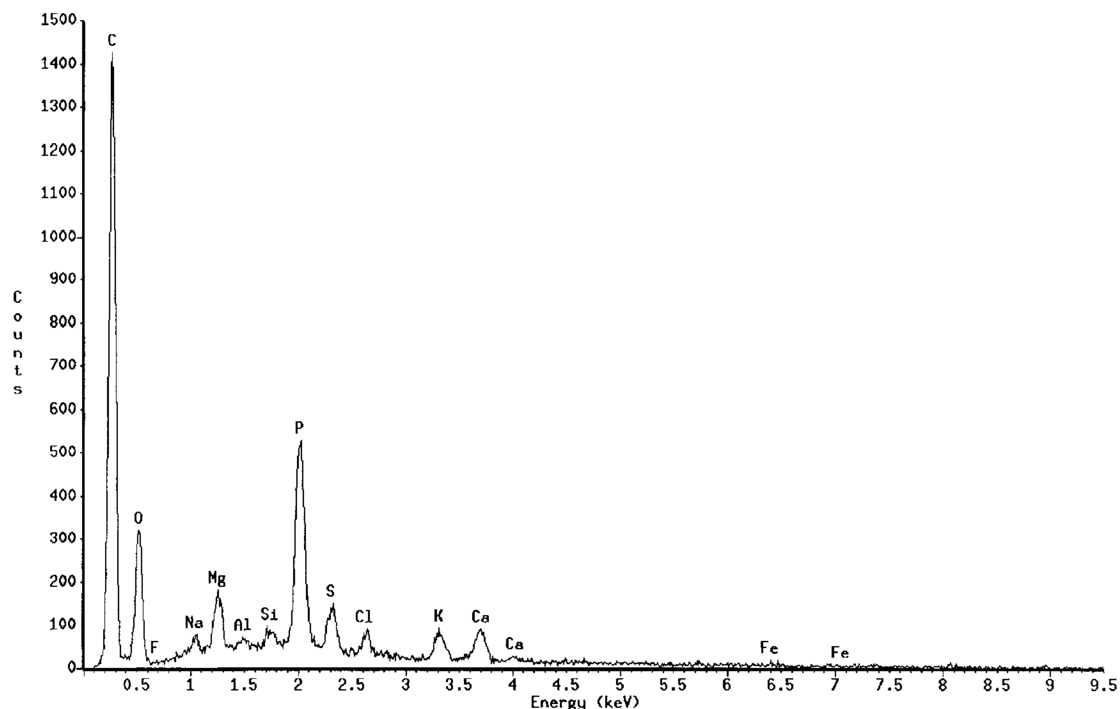

Fig. 4. Typical analytical X-ray spectrum of Daspoort EBPR anaerobic sludge EPS.

Table 2. Typical data set for elemental analysis of a cell cluster and EPS (Rooiwal}anaerobic zone). Percentages indicate elemental content of the specific field analysed. The K following every element indicates the K-shell of the specific atom

Cell clusters		EPS	
Element	Weight range (%)	Element	Weight range (%)
Na-K	0.3 – 1.5	Na-K	3.0 - 4.2
Mg-K	16.9 – 18.7	Mg-K	8.9 – 9.7
Al-K	ND[a]	Al-K	5.3 – 6.5
Si-K	ND	Si-K	8.3 – 9.1
P-K	58.4 – 61.4	P-K	34.5 – 36.5
S-K	0.2 – 1.7	S-K	11.8 – 12.9
Cl-K	0.4 – 1.8	Cl-K	1.4 – 2.2
K-K	17.1 – 20.5	K-K	11.9 – 12.9
Ca-K	ND	Ca-K	6.3 – 7.3
Fe-K	ND	Fe-K	2.7 – 4.7

CONCLUSIONS

Cell clusters with associated EPS, on average, contained between 57% and 59% phosphorus, while EPS alone contained, on average, between 27% and 30% phosphorus. Results suggest that phosphorus removal in activated sludge might be due not only to PAO, but also by EPS acting as a phosphorus reservoir.

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