QUANTIFICATION OF PHOSPHORUS IN EXTRACELLULAR POLYMERIC SUBSTANCES (EPS) ASSOCIATED WITH THE ACTIVATED SLUDGE FLOCS

by

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I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or part has been submitted at any university for a degree.

Signature: 

Date: 04/08/2003

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Summary

Several mechanisms have been proposed to explain the enhanced uptake of phosphorus by microorganisms in waste water. It has been shown that for biological phosphorus removal to occur in waste water treatment plants, biomass first needs to pass through an oxygen and nitrogen free phase, before entering the phase where an electron acceptor is present. It has been indicated that not all phosphorus removed in activated sludge systems can be accounted for by polyphosphate accumulating bacteria (PAO), the extracellular polymeric substances (EPS) also play a role in phosphorus removal. EBPR in waste water treatment has been studied for about 20 years in many countries and there are many successful full scale applications in the treatment of municipal waste water. The aim of this study was to quantify the concentration of phosphorus in the EPS and determine the chemical composition of the EPS. Activated sludge samples were collected from five different municipal waste water treatment plants situated in South Africa (Gauteng province). The phosphorus concentration was determined using the scanning electron microscopy combined with energy dispersive spectrometry (SEM/EDS). The amount of

phosphorus in activated sludge flocs plus EPS was 50.5% of the total elemental analysis on average and the EPS alone contained 25% of phosphorus on average. The anaerobic activated sludge and EPS samples contained higher concentration of orthophosphate than in the aerobic activated sludge and the aerobic EPS samples. This was due to the fact that the orthophosphate in the aerobic zone was consumed. The method for quantifying mixed liquor suspended solids (MLSS) based on freeze drying was comparable to conventional method, but had an advantage of yielding high concentration of MLSS.

Four different extraction methods, i.e. formaldehyde-NaOH, regular centrifugation (RCF), formaldehyde and EDTA were employed to study their effectiveness in extracting the extracellular polymeric substances from aerobic and anaerobic activated sludge samples. The formaldehyde-NaOH extraction procedure yielded the highest concentration of EPS as compared to the other three methods. In general, all the methods extracted about 44% protein, 33% carbohydrates and 1-3% DNA. The EPS sample contained more proteins than carbohydrates, which might be due to the fact that more exo-enzymes are entrapped in the EPS matrix. The results indicated that possible mechanism of phosphorus removal by EPS is that, phosphorus binds to cations (Mg^{2+}, K^{+} etc.) to make a insoluble compounds (Magnesium-phosphate compounds, Calcium phosphate compounds etc.) which precipitates out in the EPS associated with the activated sludge flocs. In conclusion it may be postulated that potassium, magnesium and calcium are important cations needed in phosphorus removal in terms of phosphate found in the EPS. In order to understand the mechanism of phosphorus removal by EPS further research is recommended.

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Opsoming

Verskeie meganismes is voorgestel om die vermeerderde opname van fosfor, deur mikroörganismes in afloopwater, te verklaar. Daar is bewys dat biologiese fosfor verwydering, in afloopwater behandelings aanlegte, slegs plaasvind wanneer die biomassa deur 'n suurstof/stikstof vrye fase beweeg, voordat dit die fase binnegaan waar 'n elektron akseptant teenwoordig is. Dit is duidelik dat die polifosfaat akkumuleer bakterieë (PAO), nie alleenlik verantwoordelik gehou kan word, vir die verwydering van fosfaat in geaktiveerde slyk systeme nie. Die ekstrasellulêre polimeriese stowwe (EPS) speel ook 'n rol in fosfaat verwydering. EBPR in afloopwater behandeling, is vir die afgelope 20 jaar in baie lande gestudeer en daar bestaan baie suksesvolle, groot skaalse toepassings in die behandeling van munisipale afloopwater. Die doel van hiedie studie is om die konsentrasie van fosfor in die EPS te kwantifiseer, asook om die chemiese samestelling van die EPS te bepaal. Geaktiveerde slyk monsters is versamel vanaf vyf verskillende munisipale afloopwater behandeling aanlegte, geleë in Suid-Afrika (Gauteng provinsie). Die fosfor konsentrasie is bepaal deur gebruik te maak van die skandeer

elektron mikroskoop in kombinasie met energie dispergeer spektrometrie (SEM/EDS). Die hoeveelheid fosfor in geaktiveerde slyk vlokke, tesame met EPS was gemiddeld 50.5% van die totale elementale analiese. Die EPS alleenlik bevat gemiddeld 25% fosfor. Die anaerobiese geaktiveerde slyk en EPS monsters bevat hoër konsentrasies orthofosfaat as die aerobiese monsters. Dit is as gevolg van die feit dat die orthofosfaat in die aerobiese zone verteer is. Die metode, gebaseer die vries droging, vir die kwantifiseering van mixed liquor suspended solids (MLSS) was vergelykbaar met die konfensionele metode. Hierdie vries droog metode is meer voordelig, aangesien hoë konsentrasies van MLSS opgebeur word.

Vier verskillende ekstraksie metodes nl. Formaldehied-NaOH, gewone sentrifugasie (RCF), formaldehied en EDTA. Hierdie metodes se effektiwiteit, in die ektraksie van EPS vanaf aerobiese en anaerobiese monsters, is gestudeer. In vergelyking met die ander drie metodes het formaldehied-NaOH die hoogste konsentrasie EPS opgelewer. Oor die algemeen het al vier metodes 44% proteïne, 33% koolhidrate en 1-3% DNA opgelewer. Die EPS monster het meer proteïne as koolhidrate bevat, dit mag as gevolg van die feit wees dat meer ekso-ensieme vasgevang is in die EPS matriks. Die resultate dui op 'n moontlike meganisme vir fosfor verwydering deur EPS. Die fosfor bind aan die katione (Mg^{2+}, K^{+} ens.) om onoplosbare verbindings (Magnesium-fosfaat verbindings, Kalium-fosfaat verbindings ens.) te vorm. Hierdie verbindings presipiteer uit die EPS geassosieer met die geaktiveerde slyk vlokke.

Ten slotte kan die volgende stelling gemaak word. Kalium (K^{+}), Magnesium (Mg^{2+}) en Kalsium(Ca^{2+}) is belangrike katione, nodig in die verwydering van fosfor in terme van die fosfaat gevind in die EPS. Verdere studie word aanbeveel, om die meganisme van fosfor verwydering deur EPS, te verstaan.

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