

**THE ROLE OF ACTIVATED SLUDGE  
EXTRACELLULAR POLYMERS AND  
AEROBIC BIOMASS IN THE REMOVAL  
OF PHOSPHORUS FROM WASTEWATER**

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 been submitted at any university for a degree.

Signature: *[Handwritten Signature]*

Date: 16 July 2001

*"Science has 'explained' nothing; the more we know  
the more fantastic the world becomes and the  
profounder the surrounding darkness."*

— Aldous Huxley (1894-1964), British novelist.

*"An expert is a man who has made all the mistakes which  
can be made in a very narrow field."*

— Niels Bohr (1885-1962)

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**Promoter:** Prof. T.E. Cloete  
**Department:** Microbiology and Plant Pathology  
**Degree:** M.Sc. Microbiology

## SUMMARY

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Research has indicated the relationship between biomass and phosphorus removal in activated sludge. Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) are often used as indicators of biomass, and used as such in the mathematical modelling of biological phosphorus removal. Not all phosphorus removed in activated sludge systems can be accounted for by polyphosphate accumulating organisms (PAO). The objectives of this study were to determine the relationship between the MLSS and MLVSS fractions and phosphorus removal in activated sludge, to compare these fractions, together with total plate count (TPC) and adenosine triphosphate (ATP) bacterial counts as measures of viable biomass and to investigate the role of extracellular polysaccharides (EPS) in the removal of phosphorus from wastewater. The hypothesis of the study was that the same amount of MLSS and, specifically MLVSS, of different activated sludges should show similar orthophosphate uptake abilities if these were to be indicative of biomass. To this end two experiments were conducted. In experiment 1, sterile mixed liquor growth medium was inoculated with equal amounts (40 grams) of wet sludge pellets from five different 3-stage Bardenpho activated sludge systems with similar sludge ages. In experiment 2, the MLSS and

orthophosphate concentrations in the same plants used in experiment 1 were simulated. Orthophosphate removal was determined hourly and differed amongst systems. Different orthophosphate removal capacities were attributed to differences in the MLSS active biomass fraction of the different activated sludges. Although MLSS and MLVSS showed the same trend in orthophosphate removal, initial concentrations of these fractions could not be directly linked to differences in orthophosphate uptake abilities of different sludges, indicating the unsuitability of MLSS and MLVSS as indicators of viable biomass in activated sludge. However, orthophosphate removal was consistently higher in the sludges with higher ATP and TPC values, indicating a relationship between the active biomass fraction of the MLSS and orthophosphate removal.

A method for the qualitative and quantitative *in situ* characterization of PAO cell clusters and closely associated EPS is described. X-ray microanalysis was performed on samples from four activated sludge plants. Analyses were done by means of scanning electron microscopy (SEM) combined with energy dispersive spectrometry (EDS). On average, cell clusters with associated EPS contained between 57 and 59 % phosphorus, while EPS alone contained between 23 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. Extraction of EPS from two different activated sludge plants yielded similar amounts of EPS. Comparison of EDS results before and after EPS extraction, indicated possible intracellular leakage during homogenization, while phosphorus may be complexed in localized iron and aluminium precipitates in wastewater treatment plants employing chemical treatment to attain effluent standards. These precipitates were probably removed by filtration during the extraction procedure employed.

# DIE ROL VAN GEAKTIVEERDE SLYK EKSTRASELLULÊRE POLIMERE EN AËROBE BIOMASSA IN DIE VERWYDERING VAN FOSFOR UIT AFVALWATER

deur

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## OPSOMMING

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Navorsing het die verband tussen biomassa en fosfor verwydering in geaktiveerde slyk aangetoon. Mengvloeistof gesuspendeerde soliede materiaal (MGSM) en mengvloeistof vlugtige gesuspendeerde soliede materiaal (MVGSM) word dikwels gebruik as indikators van biomassa en gebruik in die wiskundige modellering van biologiese fosfor verwydering. Nie alle fosfor verwyder in geaktiveerde slyk sisteme kan toegeskryf word aan poli-fosfaat akkumulerende organismes (PAO) nie. Die doelwitte van hierdie studie was om die verwantskap tussen die MGSM en MVGSM fraksies en fosfor verwydering in geaktiveerde slyk te bepaal, om hierdie fraksies, tesame met die totale plaat telling (TPT) en adenosien trifosfaat (ATF) bakteriese tellings as indikators van lewensvatbare biomassa te vergelyk en die rol van ekstrasellulêre polisakkariede (EPS) in die verwydering van fosfor uit afvalwater te ondersoek. Die hipotese van die studie was dat dieselfde hoeveelheid MGSM, en spesifiek, MVGSM van verskillende geaktiveerde slyke dieselfde ortofosfaat opname behoort te toon as hierdie fraksies biomassa sou aandui. Om die hipotese te toets is twee verskillende eksperimente uitgevoer. In eksperiment 1 is steriele mengvloeistof groeimedium geïnkuleer met gelyke hoeveelhede (40 g) nat slyk pille verkry uit vyf 3-fase Bardenpho geaktiveerde slyk

aanlegte met ooreenkomstige slykouderdomme. In eksperiment 2 is dieselfde geaktiveerde slyk aanlegte as in eksperiment 1 gebruik en is die MVGSM en ortofosfaat konsentrasies in die aanlegte nageboots. Ortofosfaat verwydering was uurliks bepaal en het verskil tussen die sisteme. Die verskillende ortofosfaat verwyderings kapasiteite wat waargeneem is was toegereken aan verskille in die MGSM aktiewe biomassa fraksie van die verskillende slyke. Al het MGSM en MVGSM dieselfde patroon in ortofosfaat verwydering getoon, kon aanvanklike konsentrasies van hierdie fraksies nie direk gekoppel word aan verskille in die ortofosfaat opname vermoë van die verskillende slyke nie, wat die onaanvaarbaarheid van MGSM en MVGSM as indikatore van lewensvatbare biomassa in geaktiveerde slyk aandui. Ortofosfaat verwydering was egter konsekwent hoër in die slyke met hoër ATF en TPT waardes, wat die verwantskap tussen die lewensvatbare biomassa fraksie van die MGSM en ortofosfaat verwydering aantoon.

'n Metode vir die kwalitatiewe en kwantitatiewe *in situ* karakterisering van PAO sel-trosse en naby-geassosieerde EPS word beskryf. X-straal mikroanalise was uitgevoer op monsters uit vier geaktiveerde slyk aanlegte. Analises was uitgevoer deur middel van skandeer elektron mikroskopie (SEM) gekombineer met energie verstrooiende spektrometrie (EVS). Gemiddeld, het sel-trosse met geassosieerde EPS tussen 57 en 59 % fosfor bevat, terwyl EPS alleen tussen 23 en 30 % fosfor bevat het. Resultate het voorgestel dat fosfor verwydering in geaktiveerde slyk nie net toegeskryf kan word aan PAO nie, maar ook aan EPS wat optree as a fosfor reservoir. Ekstraksie van EPS vanuit twee verskillende geaktiveerde slyk aanlegte het eenderse hoeveelhede EPS opgelewer. Vergekyking van EVS resultate voor en na EPS ekstraksie het moontlike intrasellulêre lekkasie gedurende homogenisasie aangedui, terwyl fosfor moontlik gekomplekseer met gelokaliseerde yster en aluminium presipitate mag voorkom in afvalwater behandelings aanlegte wat chemiese behandeling gebruik om uitvloei-standaarde te handhaaf. Hierdie presipitate is moontlik verwyder deur filtrasie gedurende die ekstraksie prosedure wat gebruik is.

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## LIST OF ABBREVIATIONS

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<	Smaller than
>	Larger than
%	Percent
μ	Micron
μg	Microgram
Acetoacetyl-CoA	Acetoacetyl coenzyme A
Acetyl-CoA	Acetyl coenzyme A
ADP	Adenosine diphosphate
Al <sup>3+</sup>	Aluminium ion
AMP	Adenosine monophosphate
AO	Acridine Orange
API	Analytical profile index
ATP	Adenosine triphosphate
BEPR	Biological excess phosphorus removal
Bio-P	Biological phosphorus
BNR	Biological nutrient removal
BOD <sub>5</sub>	5 day biological oxygen demand test
BPR	Biological phosphorus removal
°C	Degrees Celcius
<sup>13</sup> C	Carbon-13 isotope
<sup>14</sup> C	Radio-labelled carbon-14 isotope
Ca <sup>2+</sup>	Calcium ion
CaCO <sub>3</sub>	Calcium carbonate
cal	Calories
cap	Capita
cells.ml <sup>-1</sup>	Cells per millilitre
cfu	Colony forming units
CGY	Casitone glycerol yeast autolysate

$^{14}\text{CO}_2$ :	Radio-labelled carbon dioxide
$\text{CO}_2$ :	Carbon dioxide
COD:	Chemical Oxygen Demand
CLSM:	Confocal laser scanning microscopy
d:	Day/s
DAPI:	4'6-diamidino-2-phenylindole
DNA:	Deoxyribonucleic acid
DO:	Dissolved oxygen
EBPR:	Enhanced biological phosphate removal
EDS:	Energy dispersive spectroscopy
EDTA:	Ethylenediaminetetraacetic acid
Eh:	Redox potential
EM:	Electron microscopy
EPS:	Extracellular polysaccharides/extracellular polymeric substances
ESEM:	Environmental scanning electron microscope
EUB:	Eubacterial probe
FDA:	Fluorescein diacetate
$\text{Fe}^{3+}$ :	Ferric iron ion
FISH:	Fluorescent <i>in situ</i> hybridization
FITC:	Fluorescein isothiocyanate
FT-IR:	Infrared spectroscopy
G6PDH:	Glucose-6-phosphate dehydrogenase
g:	Gram
GAO:	Glycogen accumulating organisms
GC:	Gas chromatography
GC-MS:	Gas chromatography – mass spectroscopy
$\text{g}\cdot\text{g}^{-1}$ :	Gram per gram
$^1\text{H}$ :	Hydrogen-1 isotope
$\text{H}^+$ :	Hydrogen ion
$\text{H}_2\text{O}$ :	Water

H <sub>2</sub> S:	Hydrogen sulfide
H <sub>2</sub> SO <sub>4</sub>	Sulphuric acid
h:	Hour
HPLC:	High performance liquid chromatography
HPO <sub>4</sub> <sup>2-</sup> :	Hydrogen phosphate ion
H <sub>2</sub> PO <sub>4</sub> <sup>-</sup> :	Dihydrogen phosphate ion
H <sub>3</sub> PO <sub>4</sub> :	Phosphoric acid
K <sup>+</sup> :	Potassium ion
kg:	Kilogram
kcal:	kilocalories
kJ:	Kilojoule
KH <sub>2</sub> PO <sub>4</sub> :	Potassium dihydrogen phosphate
KNO <sub>3</sub> :	Potassium nitrate
l:	Liter
M:	Molar
MCRT:	Mean cell residence time
Mg <sup>2+</sup> :	Magnesium ion
MgSO <sub>4</sub> ·7H <sub>2</sub> O:	Magnesium sulphate heptahydrate
mg:	Milligram
mg.g <sup>-1</sup> :	Milligram per gram
mg.l <sup>-1</sup> :	Milligram per litre
min:	Minutes
ml:	Millilitre
MLOSS:	Mixed liquor organic suspended solids
MLSS:	Mixed liquor suspended solids
MLVSS:	Mixed Liquor Volatile Suspended Solids
MPN:	Most Probable Number
MRNA:	Messenger RNA
N:	Nitrogen
N <sub>2</sub> :	Nitrogen gas

N <sub>2</sub> O:	Nitrous oxide
NA:	Nutrient Agar
NaC <sub>2</sub> H <sub>3</sub> O <sub>2</sub> :	Sodium acetate
NaCl:	Sodium chloride
NAD:	Nicotinamide adenine dinucleotide
NaOH:	Sodium hydroxide
Na <sub>2</sub> SO <sub>4</sub> :	Sodium sulphate
NaS <sub>2</sub> O <sub>3</sub> :	Sodium thiosulphate
ND:	Not determined
NH <sub>3</sub> :	Ammonia
NH <sub>4</sub> <sup>+</sup> :	Ammonium ion
NH <sub>3</sub> -N:	Ammonia nitrogen
nm:	Nanometer
NMR:	Nuclear magnetic resonance spectroscopy
NO <sub>2</sub> <sup>-</sup> :	Nitrite ion
NO <sub>3</sub> <sup>-</sup> :	Nitrate ion
NO <sub>3</sub> -N:	Nitrate nitrogen
NO <sub>x</sub> :	Nitrogen oxides
O <sub>2</sub> :	Oxygen
OH <sup>-</sup> :	Hydroxide ion
OUR:	Oxygen utilization rate
P:	Phosphorus
PAO:	Polyphosphate accumulating organisms
PCR:	Polymerase chain reaction
pH:	Hydrogen ion concentration
PHA:	Poly-β-hydroxyalkanoates
PHB:	Poly-β-hydroxybutyrate
PO <sub>4</sub> <sup>3-</sup> :	Orthophosphate ion
Poly-P:	Polyphosphate
R:	Reactive group/substituent

RBCOD:	Readily biodegradable chemical oxygen demand
rpm:	Revolutions per minute
RNA:	Ribonucleic acid
rRNA:	Ribosomal ribonucleic acid
S <sup>0</sup> :	Elemental sulphur
S <sub>2</sub> <sup>-</sup> :	Sulphide ion
SCFA:	Short chain fatty acids
SDS-PAGE:	Sodium dodecyl sulphate polyacrylamide electrophoresis
SEM:	Scanning electron microscopy
SO <sub>4</sub> <sup>2-</sup> :	Sulphate ion
SRB:	Sulphate reducing bacteria
SS:	Suspended solids
TCA:	Tricarboxylic acid
TEM:	Transmission electron microscopy
TKN:	Total Kjeldahl nitrogen
tRNA:	Transfer RNA
TOC:	Total organic carbon
TP:	Total phosphorus
TPC:	Total plate counts
T <sub>x</sub> :	Time x
UCT:	University of Cape Town
VFA:	Volatile fatty acids
VSS:	Volatile suspended solids
WTP:	Wastewater treatment plant/s
Yr:	Year