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

by

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Submitted in partial fufillment of the requirements for the degree

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In the Faculty of Natural and Agricultural Science

Department of Microbiology and Plant Pathology
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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: _____

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

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.

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DIE ROL VAN GEAKTIVEERDE SLYK EKSTRASELLULêRE POLIMERE EN AëROBE BIOMASSA IN DIE VERWYDERING VAN FOSFOR UIT AFVALWATER

deur

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Graad: M.Sc. Mikrobiologie

OPSOMMING

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 geïnokuleer 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 indikators 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 uitvevoer deur middel van skandeer elektron mikroskopie (SEM) gekombineer met energie verstrooïende spektrometrie (EVS). Gemiddeld, het sel-trosse met geassosieerde EPS tusen 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. Vergeyking van EVS resultate voor en na EPS ekstraksie het moontlike intrasellulêre lekkasie gedurende homogenisasie aangedui, terwyl fosfor moontlik gekomplekseerd met gelokaliseerde yster en aluminium presipitate mag voorkom in afvalwater behandelings aanlegte wat chemiese beandeling 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

<:	Smaller than
>:	Larger than
%:	Percent
µ:	Micron
µg:	Microgram
Acetoacetyl-CoA:	Acetoacetyl coenzyme A
Acetyl-CoA:	Acetyl coenzyme A
ADP:	Adenosine diphosphate
Al^{3+}:	Aluminium ion
AMP:	Adenosine monophosphate
AO:	Acridine Orange
API:	Analytical profile index
ATP:	Adenosine triphosphate
BEPR:	Biological excess phosphorus removal
Bio-P:	Biological phoshorus
BNR:	Biological nutrient removal
BOD5:	5 day biological oxygen demand test
BPR:	Biological phosphorus removal
°C:	Degrees Celcius
^{13}C:	Carbon-13 isotope
^{14}C:	Radio-labelled carbon-14 isotope
Ca^{2+}:	Calcium ion
$CaCO_3$:	Calcium carbonate
cal:	Calories
cap:	Capita
cells.ml^{-1}:	Cells per millilitre
cfu:	Colony forming units
CGY:	Casitone glycerol yeast autolysate

$^{14}CO_2$:	Radio-labelled carbon dioxide
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
Fe^{3+}:	Ferric iron ion
FISH:	Fluorescent *in situ* 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
$g.g^{-1}$:	Gram per gram
^1H:	Hydrogen-1 isotope
H^+:	Hydrogen ion
H_2O:	Water

H_2S:	Hydrogen sulfide
H_2SO_4	Sulphuric acid
h:	Hour
HPLC:	High performance liquid chromatography
HPO_4^{2-}:	Hydrogen phosphate ion
$H_2PO_4^-$:	Dihydrogen phosphate ion
H_3PO_4:	Phosphoric acid
K^+:	Potassium ion
kg:	Kilogram
kcal:	kilocalories
kJ:	Kilojoule
KH_2PO_4:	Potassium dihydrogen phosphate
KNO_3:	Potassium nitrate
l:	Liter
M:	Molar
MCRT:	Mean cell residence time
Mg^{2+}:	Magnesium ion
$MgSO_4.7H_2O$:	Magnesium sulphate heptahydrate
mg:	Milligram
$mg.g^{-1}$:	Milligram per gram
$mg.l^{-1}$:	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_2:	Nitrogen gas

N_2O:	Nitrous oxide
NA:	Nutrient Agar
$NaC_2H_3O_2$:	Sodium acetate
NaCl:	Sodium chloride
NAD:	Nicotinamide adenine dinucleotide
NaOH:	Sodium hydroxide
Na_2SO_4:	Sodium sulphate
NaS_2O_3:	Sodium thiosulphate
ND:	Not determined
NH_3:	Ammonia
NH_4^+:	Ammonium ion
NH_3-N:	Ammonia nitrogen
nm:	Nanometer
NMR:	Nuclear magnetic resonance spectroscopy
NO_2^-:	Nitrite ion
NO_3^-:	Nitrate ion
NO_3-N:	Nitrate nitrogen
NO_x:	Nitrogen oxides
O_2:	Oxygen
OH^-:	Hydroxide ion
OUR:	Oxygen utilization rate
P:	Phosphorus
PAO:	Polyphosphate accumulating organisms
PCR:	Polymerase chain reaction
pH:	Hydrogen ion concentration
PHA:	Poly-ß-hydroxyalkanoates
PHB:	Poly-ß-hydroxybutyrate
PO_4^{3-}:	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^0:	Elemental sulphur
S_2^-:	Sulphide ion
SCFA:	Short chain fatty acids
SDS-PAGE:	Sodium dodecyl sulphate polyacrylamide electrophoresis
SEM:	Scanning electron microscopy
SO_4^{2-}:	Sulphate ion
SRB:	Sulphate reducing bacteria
SS:	Suspended solids
TCA:	Tricarboxylic acid
TEM:	Transmission electron microscopy
TKN:	Total Kjelldahl nitrogen
tRNA:	Transfer RNA
TOC:	Total organic carbon
TP:	Total phosphorus
TPC:	Total plate counts
T_x:	Time x
UCT:	University of Cape Town
VFA:	Volatile fatty acids
VSS:	Volatile suspended solids
WTP:	Wastewater treatment plant/s
Yr:	Year