Multistage and multiple biomass approaches to efficient biological nitrogen removal using biofilm cultures

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This thesis is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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April 2008

I hereby declare that this thesis is my own account of my research and contains as its main content work that has not previously been submitted for a degree at any university.

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Nitrogen is nitrogen, it passes miraculously from the air into plants, from these into animals, and from animals to us; when its function in our body is exhausted, we eliminate it, but it still remains nitrogen, aseptic, innocent.

Primo Levi in *The Periodic Table*

To Dean, thankyou

ABSTRACT

Nitrogen removal from wastewater is important for the prevention of significant health and environmental impacts such as eutrophication. Nitrogen removal is achieved by the combined action of nitrification and denitrification. Nitrification is performed by autotrophic, slow growing microorganisms that require oxygen and are inhibited in the presence of denitrifiers when oxygen and COD are available due to competition for oxygen. Denitrification however, performed by relatively fast growing heterotrophic bacteria, is inhibited by oxygen and requires COD. This implies that nitrification and denitrification are mutually exclusive. The supply of oxygen to a fresh wastewater, high in ammonia and COD, causes waste of both oxygen and COD. Conservation of COD is therefore critical to efficient wastewater treatment. The approach investigated in this study to achieve complete nitrogen removal was to physically separate the nitrification and denitrification biomasses into separate bioreactors, supplying each with appropriate conditions for growth and activity.

A storage driven denitrification sequencing batch biofilm reactor (SDDR) was established which exhibited a high level of COD storage (up to 80% of influent COD) as poly-β-hydroxybutyrate capable of removing >99% of nitrogen from wastewaters with a C/N ratio of 4.7 kg COD/kg $N–NO_3^-$. The SDDR was combined in sequential operation with a nitrification reactor to achieve complete nitrogen removal. The multiple stage, multiple biomass reactor was operated in sequence, with Phase 1 - COD storage in the storage driven denitrification biofilm; Phase 2 - ammonia oxidation in the nitrification reactor; and Phase 3 - nitrate reduction using the stored COD in the storage driven denitrification reactor. The overall rate of nitrogen removal observed was up to

1.1 mmole NH_3 L^{-1} h^{-1} and >99% of nitrogen could be removed from wastewaters with a low C/N ratio of 3.9 kg COD/kg $N–NH_3$.

The multiple stage, multiple biomass system was limited in overall nitrogen removal the reduction in pH caused by nitrification. A parallel nitrification-denitrification (PND) reactor was developed in response to the pH control issue. The PND reactor was operated with Phase 1 – COD storage in the storage driven denitrification biofilm and Phase 2 – simultaneous circulation of reactor liquor between the denitrification and nitrification biofilms to achieve complete nitrogen removal and transfer of protons. The PND reactor performed competitively with the multistage reactor (removal of >99% nitrogen from wastewaters with feed ratios of 3.4 kg COD/kg $N–NH_3$) without the need for addition of buffering material to moderate the pH.

Contents

Acknowledgements

I would like to acknowledge the support of my supervisor, Dr Ralf Cord-Ruwisch throughout this project. In addition, I would like to acknowledge the financial support of an Australian Postgraduate Award and a Murdoch Excellence Award.

Abbreviations

Anammox	Anaerobic AMMonium Oxidation
AOB	Ammonia (ammonium) oxidising bacteria
APSBBR	Alternating pumped sequencing batch biofilm reactor
AUP	Acetate uptake phase
BNR	Biological nitrogen removal
BOD	Biochemical oxygen demand
c_L	DO concentration
C/N feed	Ratio of carbon (expressed as kg COD) to nitrogen (expressed as kg N) in the feed
C/N ratio of removal	Ratio of amount of carbon consumed (expressed as kg COD) to amount of nitrogen consumed (expressed as kg N) in a trial
COD	Chemical oxygen demand (see Appendix A for derivation). In this thesis, COD is mainly used to reflect the biodegradable COD in the feed
CANON	Completely autotrophic nitrogen removal over nitrite
c_S	Saturation DO concentration
DNP	Denitrification phase
DO	Dissolved oxygen
EBPR	Enhanced biological phosphate removal
EPS	Extracellular polymeric substances
ETC	Electron transport chain
FISH	Fluorescence *in situ* hybridisation
GAO	Glycogen accumulating organisms
GC	Gas chromatography
k_La	Oxygen mass transfer coefficient (h^{-1})
K_m	Michaelis-Menten constant
NOB	Nitrite oxidising bacteria
NO_x	Collective nitrogen oxide species
ORP	Oxidation reduction potential
OUR	Oxygen uptake rate
PAO	Poly phosphate accumulating organisms

PHA	Poly hydroxyalkanoate
PHB	Poly-β-hydroxybutyrate
pK_a	Negative logarithm (base 10) of the acid dissociation constant
PND	Parallel nitrification-denitrification
SBR	Sequencing batch reactor
SND	Simultaneous nitrification-denitrification
SOUR	Specific oxygen uptake rate
TCA	Tricarboxylic acid cycle
TF	Trickling filter
TKN	Total Kjeldahl nitrogen
TS	Total solids
VFA	Volatile fatty acids
V_{max}	Maximum biochemical reaction velocity
VS	Volatile solids
WWTP	Wastewater treatment plant