Biological Phosphorus Removal by Microalgae in Waste Stabilisation Ponds

A thesis presented in partial fulfilment of the requirements for the degree of

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Abstract

Waste stabilisation ponds (WSP) are an important wastewater treatment technology used by thousands of communities around the world. Unfortunately, phosphorus removal in WSP is generally low and inconsistent. The aim of this work was to investigate biological phosphorus removal by microalgae in WSP. Luxury uptake of phosphorus, which is the accumulation of polyphosphate, is known to occur in microalgae in natural systems such as lakes; however, this mechanism has not previously been studied under WSP conditions. Three methods were used in the laboratory to investigate luxury uptake and it was shown for the first time that luxury uptake of phosphorus can occur in microalgae under typical WSP conditions. Acid-insoluble polyphosphate (AISP) is a form of phosphorus storage and acid soluble polyphosphate (ASP) is used for synthesis of cellular constituents. However, the findings of this thesis indicate that ASP may also act as a form of short term storage. The environmental factors influencing luxury uptake were investigated using laboratory experiments conducted under controlled conditions. The key environmental factors were the phosphate concentration in the wastewater, light intensity and temperature. A higher phosphate concentration increased the amount of ASP accumulation and also resulted in AISP being stored within the cells instead of being consumed for growth. Higher light intensity increased ASP accumulation, but as a consequence of elevated growth, the ASP was rapidly consumed. Temperature influenced the rate of AISP accumulation and little if any was accumulated at low temperatures. The fate of polyphosphate in the sludge layer was also studied and it was shown that polyphosphate was degraded resulting in phosphate release. Therefore, to maximise phosphorus removal the microalgae needs to be harvested. Field work showed that at times the biomass contained almost four times the amount of phosphorus required for growth which confirms that luxury uptake does indeed occur in full-scale WSP. To improve phosphorus removal in WSP both luxury uptake and the biomass concentration need to be maximised simultaneously. With this new understanding of biological phosphorus removal in WSP and the key environmental factors required it may be possible to develop a new phosphorus removal process utilising luxury uptake by microalgae.

Acknowledgements

First to my supervisors Prof. Andy Shilton, Dr. Steven Pratt and Prof. Yusuf Chisti for their help, guidance and motivation throughout my research. Their support during my PhD and all the valuable feedback on the many drafts of the research papers is greatly appreciated.

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Thanks must also go to all the staff and other postgraduate students at the Centre for Environmental Technology and Engineering during my PhD for their valuable suggestions and ideas during the completion of my experimental work. In particular I wish to thank Mr. David Miller, Mr. Kenny Wong, Ms. Selina Zhang, Mr. Leon Chen, Mr. Alistair Broughton and Dr. Ibrahim Elmetri.

Finally thank you to all my friends and family for their support during my PhD.

Table of contents

Structure of the thesis

The results and discussion chapters of this thesis are presented as a series of scientific papers. These papers have either been accepted for publication or submitted for review. Consequently there is some repetition in these chapters particularly in the introduction and methods sections. To reduce repetition the introduction sections of the papers have been shortened. A preface is included for each of these chapters to help link the chapters together and illustrate how each of these chapters contribute to investigating the objectives of this thesis.

The content of the chapters is the same as the published paper they are based on; however, some formatting changes have been made to ensure consistent style within the thesis. For example the labels for the Figures and Tables have been modified to include the chapter number (eg Figure 2 changed to Figure 3.2). The chapters have also had some minor editing for improved clarity. Where the published papers refer to other papers within the thesis, these references have been changed to the relevant chapter within the thesis. A summary of the main findings of the research presented in the publications is then given in Chapter 8.

The structure of this thesis complies with the Massey University guidelines given in the Doctoral Handbook, 2008.

List of papers and contribution

A number of chapters in this thesis are based on papers that have been accepted for publication in international peer reviewed scientific journals or presentation at peer reviewed conferences. A list of the chapters and relevant publications is given below.

Chapter 1

Parts of this chapter are based on the introduction from the following publication:

Powell, N., Shilton, A., Chisti, Y., & Pratt, S. (2009). Towards a luxury uptake process via microalgae – Defining the polyphosphate dynamics. *Water Research, 43(17), 4207-4213.*

Chapter 2

Powell, N., Shilton, A., Pratt, S., Chisti, Y., & Grigg, N. (2006). Factors effecting biological phosphorus removal in waste stabilisation ponds – A statistical analysis. *Paper presented at the 7th IWA Specialist Conference on Waste Stabilisation Ponds, Bangkok, Thailand.*

Chapter 3

Powell, N., Shilton, A., Pratt, S., & Chisti, Y. (2006). Luxury uptake of phosphorus by microalgae in waste stabilisation ponds. In R. Stuetz & L. Teik-Thye (Eds.), *Young Researchers 2006 (Water and Environment Management Series no. 12)* (pp. 249-256). London: IWA Publishing.

Chapter 4

Powell, N., Shilton, A., Pratt, S., & Chisti, Y. (2008). Factors influencing luxury uptake of phosphorus by microalgae in waste stabilisation ponds. *Environmental Science and Technology, 42(16), 5958-5962.*

Chapter 5

Powell, N., Shilton, A., Chisti, Y., & Pratt, S. (2009) Towards a luxury uptake process via microalgae – Defining the polyphosphate dynamics. *Water Research, 43(17), 4207-4213.*

Chapter 6

Powell, N., Shilton, A., Pratt, S., & Chisti, Y. Phosphate release from waste stabilisation pond sludge – Significance and fate of polyphosphate. *To be submitted.*

Chapter 7

Powell, N., Shilton, A., Chisti, Y., & Pratt, S. (2009). Luxury uptake of phosphorus by microalgae in full-scale waste stabilisation ponds. *Paper presented at the Water New Zealand Conference, Water 2020: From fragmentation to efficiency, Rotorua.*

All the research that these papers are based on was conducted during my PhD. While the papers were completed with advice and editing from my supervisors Prof. Andy Shilton, Dr. Steven Pratt, and Prof. Yusuf Chisti I designed the experiments, conducted all experimental work, analysed the results and was lead author on all the papers.

Chapter 1

Introduction

Parts of this chapter are based on the introduction section from the following publication:

Powell, N., Shilton, A., Pratt, S., & Chisti, Y. (2009). Towards a luxury uptake process via microalgae - Defining the polyphosphate dynamics. *Water Research, 43(17), 4207-4213*

Waste stabilization ponds (WSP) are used for wastewater treatment by thousands of small communities around the world. These ponds offer an appropriate wastewater treatment technology for small communities as they are simple to construct and inexpensive to operate.

The classic relationship between microalgae and bacteria in WSP is illustrated in Figure 1.1. Microalgae photosynthesise which requires sunlight, carbon dioxide and nutrients and produces new cells and oxygen. The oxygen is then utilised by the aerobic bacteria to oxidise organic matter. During bacterial respiration carbon dioxide is produced which is then consumed by the microalgae during photosynthesis. The mutualistic relationship shown in Figure 1.1 does however simplify the complex interactions that occur between the microalgae and bacteria in WSP. For example bacterial growth can reduce light penetration which subsequently influences microalgal growth. Bacteria can also influence microalgal growth by producing both growth promoting (de-Bashan et al., 2004) and growth limiting substances (Rhee, 1972).

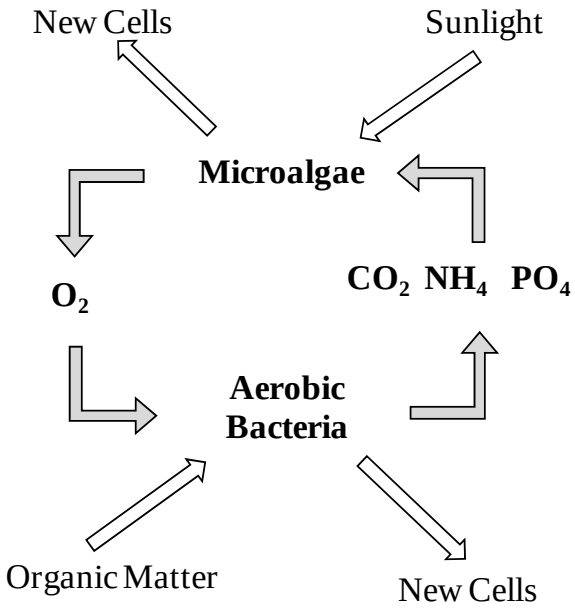

Figure 1.1: Mutualistic relationship between microalgae and bacteria in WSP

WSP provide effective wastewater treatment in terms of organic carbon and pathogen removal. However, phosphorus removal in WSP is often low, generally between 15 and 50% (Picot et al., 1992; Racault et al., 1995; Garcia et al., 2000). Phosphorus removal from wastewater is important as high concentrations of phosphate can cause

eutrophication of rivers and lakes that ultimately receive the treated wastewater. Consequently, regulators are imposing stricter standards resulting in growing pressure on treatment plants to upgrade their systems to improve phosphorus removal.

There are two options for improving phosphorus removal in small communities currently using WSP. These are to upgrade the existing ponds or to replace the WSP with another wastewater treatment process. One of the most common upgrade options is to add chemical dosing. Chemicals such as alum, ferric chloride and polymers can be added to precipitate out the phosphate ions. The precipitates are then removed via sedimentation. While chemical dosing does offer effective phosphorus removal, it significantly increases the cost and complexity of the pond treatment system and the large quantities of chemical sludge that are produced can prove difficult to dispose of.

The other option is to simply replace WSP with another wastewater treatment process that is capable of high phosphorus removal. However, if WSP are replaced the hundreds of millions of dollars already invested into building pond systems by thousands of communities around the world will be lost. Replacement of WSP would also represent the loss of a simple and appropriate technology. This is a significant concern because it was the simple and sustainable nature of ponds which made them so popular and widespread to begin with.

A technology which can achieve high phosphorus removal is the enhanced biological phosphorus removal (EBPR) form of the activated sludge process which utilises polyphosphate accumulation by bacterial biomass. However, compared to WSP, EBPR requires relatively large amounts of energy, requires on-going operator control to operate efficiently and has significant capital and operating costs that many small communities accustomed to the simplicity of ponds will struggle to afford.

Phosphorus removal was also once a problem for communities served by activated sludge systems until the EBPR process was developed. It may therefore be feasible that a similar solution could be found for WSP by investigating the phosphorus uptake dynamics that naturally occur in microalgae.

Growth of microalgae in WSP consumes phosphorus as it is an essential element required for making cellular constituents such as phospholipids, nucleotides and nucleic acids (Miyachi et al., 1964). Microalgae typically contain approximately 1% phosphorus by dry weight (Borchardt & Azad, 1968; Hemens & Mason, 1968; Azad & Borchardt, 1970; Goldman, 1980; Kaplan et al., 1986). This is determined by evaluating microalgal growth over a range of phosphate concentrations. The minimum phosphate concentration which is not growth limiting is referred to as the critical concentration of phosphorus (Azad & Borchardt, 1970). At this critical concentration the biomass is analysed for total phosphorus content and several algal species have shown 'remarkable consistency in their chemical composition' (Goldman, 1980). However, under certain conditions microalgae can be triggered to take up much more phosphorus than is necessary for survival. This extra phosphorus is stored as polyphosphate for use as an internal resource when the external phosphorus concentration is limiting for growth (Kuhl, 1974). Polyphosphates are long chain molecules of variable length with the general structure shown in Figure 1.2. Polyphosphates are generally divided into two types according to the extraction method used. These are acid-soluble polyphosphate and acid-insoluble polyphosphate. Acid-soluble polyphosphates are thought to be shorter chain molecules and involved in metabolism (Kuhl, 1962) while acid-insoluble polyphosphate are longer chain polyphosphates which are used for phosphorus storage (Miyachi et al., 1964).

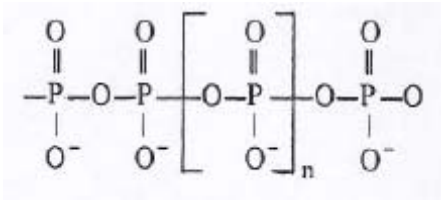

Figure 1.2: Structure of polyphosphate (Kaplan et al., 1986)

Previous research on polyphosphate accumulation in microalgae has largely been limited to its occurrence in natural ecosystems such as lakes and rivers. This work has shown that two different mechanisms are involved in the storage of polyphosphate in microalgae. When microalgae are starved of phosphorus and then re-exposed to it, the consequent storage is referred to as 'over-compensation' (Aitchison & Butt, 1973; Chopin et al., 1997), or the 'overshoot phenomenon' (Cembella et al., 1984). Over-

compensation occurs in natural systems such as lakes where microalgae encounter periods of phosphorus starvation. The other polyphosphate storage mechanism is referred to as ‘luxury uptake’. Luxury uptake of phosphorus in microalgae is defined as when the microalgae contain more phosphorus than required for growth without a prior starvation stage (Eixler et al., 2006). In WSP the prevailing high nutrient concentrations mean that periods of phosphorus starvation are unlikely and any polyphosphate accumulation will therefore predominately be due to the luxury uptake mechanism. While many researchers have studied the effect of starvation (for example Borchardt & Azad, 1968; Aitchison & Butt, 1973; Gotham & Rhee, 1981; Jansson, 1993) the factors which influence luxury uptake are poorly understood. Luxury uptake by microalgae has not previously been studied under the conditions found in WSP and has otherwise been overlooked in regard to its potential as a phosphorus removal technique for WSP.

The ability of microalgae to carry out luxury uptake of phosphorus has only been investigated for a limited number of microalgal species. Microalgae that have been shown to be capable of luxury uptake such as *Scenedesmus* (Azad & Borchardt, 1969; Keenan & Auer, 1974) and *Chlorella* (Borchardt & Azad, 1968; Keenan & Auer, 1974) are known to occur in WSP (Pearson, 2005). However, the presence of these species of microalgae does not necessarily mean that luxury uptake occurs as the environmental conditions must also be present to trigger polyphosphate accumulation.

To improve net phosphorus removal in WSP the sludge layer must also be considered as phosphorus removal is dependent on the amount of phosphorus retained in the sludge. Phosphorus enters the sludge as a result of chemical precipitation and biomass settling. However, some this phosphorus is subsequently released back into the overlying wastewater. Therefore, the rate of phosphate release from the sludge and whether polyphosphate is released from the settled microalgal biomass must also be considered.

With further understanding of the luxury uptake mechanism and the key environmental factors required, it may be possible to upgrade or redesign WSP optimised for biological phosphorus removal by microalgae.

The general scope of this work was to investigate biological phosphorus removal by microalgae in WSP while the more specific objectives were to:

1. Determine the key environmental factors that influence biological phosphorus uptake by microalgae under the conditions that typically occur in WSP.

2. Determine whether luxury uptake of phosphorus occurs in microalgae under the conditions that typically occur in WSP.

3. Investigate how key environmental factors that have been identified influence the luxury uptake mechanism in microalgae under the conditions that typically occur in WSP.

4. Determine the rate of phosphate release from WSP sludge and investigate whether polyphosphate is released.

5. Investigate the significance of biological phosphorus uptake and determine whether luxury uptake occurs in full scale WSP.

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Chapter 2

Screening of influencing factors

Chapter preface

The first objective of this thesis was to determine the key environmental factors that influence biological phosphorus uptake by microalgae under the conditions that typically occur in WSP. In this chapter screening experiments were used to investigate the influence of the phosphate concentration, nitrogen concentration, light intensity, diurnal cycle and temperature on biological phosphorus uptake and biomass growth. The levels of these different parameters were chosen to reflect the typical conditions found in WSP. As a result of these experiments the key environmental factors influencing biological phosphorus uptake were identified.

This chapter is based on the following publication:

Powell, N., Shilton, A., Pratt, S., Chisti, Y., & Grigg, N. (2006). *Factors effecting biological phosphorus removal in waste stabilisation ponds – A statistical analysis.* A peer reviewed paper presented at the 7th IWA Specialist Conference on Waste Stabilisation Ponds, Bangkok, Thailand.

Abstract

This chapter reports the systematic testing of how five factors, each at two levels, influence biological phosphorus uptake. The factors tested were the phosphorus concentration, nitrogen concentration, light intensity, diurnal light cycle and temperature. Batch reactors, inoculated with waste stabilisation pond microalgae and fed with synthetic wastewater containing chelating chemicals to prevent precipitation, were used for the study. Factorial experimental design resulted in 16 separate experiments being conducted and then analysed to determine which factors were statistically significant. It was found that the relationship between biological phosphorus uptake and (i) phosphorus concentration, (ii) light intensity and (iii) temperature were all statistically significant to a 95% confidence interval. Further analysis of results suggested that biomass growth was not the only biological phosphorus uptake mechanism occurring. It appeared that a large amount of phosphorus uptake was due to some other mechanism which was hypothesised to be luxury uptake by the microalgae.

2.1 Introduction

Phosphorus removal in waste stabilisation ponds (WSP) occurs as a result of both chemical and biological mechanisms. Precipitation occurs due to an elevated pH resulting from algal growth. The phosphate ions precipitate with cations such as calcium, iron and magnesium present in the wastewater. Phosphorus is also removed biologically as it is a nutrient required for organism growth. However, biological phosphorus removal in WSP and the mechanisms responsible are not well understood.

With improved understanding of the phosphorus removal mechanisms in WSP it is possible that ponds could be better designed for phosphorus removal. Without such improvements many pond systems face becoming obsolete, thereby exposing the communities they serve to significant capital upgrades and the subsequent loss of the simple, low cost operation for which ponds are renowned.

This chapter reports on a systematic evaluation to determine which factors have a statistically significant effect on biological phosphorus uptake in WSP microalgae.

2.2 Methods

Batch experiments were conducted using one litre Erlenmeyer flasks that were mixed using magnetic stirrers. Fluorescent daylight lamps were used to simulate day and night (Philips, 36W). All experiments were conducted in constant temperature rooms. A synthetic wastewater medium was used according to Davis and Wilcomb (1967) which contained the metal ion chelating agents EDTA (ethylenediaminetetraacetate) and sodium citrate to prevent precipitation thereby allowing the biological mechanisms to be studied. The absence of precipitation was confirmed by increasing the pH and measuring minimal change in the soluble phosphate concentration. The composition of the synthetic wastewater medium is given in Tables 2.1 and 2.2. This medium contained no readily degradable organic carbon to limit bacterial growth as bacterial growth may have influenced factors such as the light intensity the microalgae were exposed to because of shading effects. Possible implications of this are discussed further in Chapter 8.

Table 2.1: Composition of the synthetic wastewater medium

Chemical	**Concentration (mg/l)**
$Ca(NO_3)_2$	60
KNO_3	70
NH_4Cl	57
KH_2PO_4	20
Na_2SiO_3	20
NaCl	70
$MgSO_4$	20
$NaHCO_3$	125
$Na_3C_6H_5O_7$	250
Hutners Trace elements	1 ml/L

Table 2.2: Composition of Hutners trace elements used in the synthetic wastewater medium

Chemical	Concentration (g/l)
EDTA	5
$ZnSO_4$	2
H_3BO_3	1
$CaCl_2$	0.662
$MnCl_2$	0.5
$FeSO_4$	0.5
$CoCl_2$	0.15
$CuSO_4$	0.15
$(NH_4)_6Mo_7O_{24}$	0.1

A culture from a facultative WSP in Ashhurst, New Zealand, which was dominated by *Scenedesmus* sp. was used to start a continuous culture inoculum tank which is discussed further in Chapter 3. All experiments were inoculated from this culture to ensure consistency between experiments.

The phosphate concentration in the liquid and the biomass concentration measured as dry weight were measured in duplicate for each batch reactor at the start of the experiment and at days 1, 3 and 7. The dry weight was measured according to Lee and Shen (2004) and the phosphate was measured using ion chromatography (Dionex ICS-2000; Dionex Corporation, Sunnyvale, CA, USA) in duplicate. Dionex IonPac AS11-HC (4 × 250mm) and Dionex IonPac AG11-HC columns were used with a 25 µL sample loop. The eluent concentration was 32 mM KOH at a flow rate of 1 mL/minute and the operating temperature was 30 °C. A Suppressed Conductivity ASRS-ULTRA suppressor was used in recycle mode. The light intensity was monitored just above the surface of the reactors using a PAR (photosynthetically active radiation) irradiance sensor (QSL-2101; Biospherical Instruments, San Diego, CA, USA).

2.2.1 Design of experiments

The statistical computer program MINITAB was used to design the factorial matrix and then analyse the results. The use of factorial experimental design means that many factors can be tested at the same time, so a minimum number of experiments are required. Interactions between factors can also be tested, as two factors may not have a significant effect individually but the way they interact may be statistically significant. The factorial matrix consisted of 16 experiments each with four measurements resulting in a total of 128 data points for phosphate concentration and dry weight. The factors tested are shown in Table 2.3 along with the levels for each factor.

Table 2.3: Experimental matrix with levels of factors tested

Experiment	Phosphorus concentration (mgP/L)	Nitrogen concentration (mgN/L)	Light intensity (µE/m^2.s)	Diurnal light cycle (hrs on/off)	Temperature (°C)
A	5	35	60	9/15	25
B	10	35	60	9/15	15
C	5	70	60	9/15	15
D	10	70	60	9/15	25
E	5	35	150	9/15	15
F	10	35	150	9/15	25
G	5	70	150	9/15	25
H	10	70	150	9/15	15
I	5	35	60	15/9	15
J	10	35	60	15/9	25
K	5	70	60	15/9	25
L	10	70	60	15/9	15
M	5	35	150	15/9	25
N	10	35	150	15/9	15
O	5	70	150	15/9	15
P	10	70	150	15/9	25

Four experimental runs were undertaken each with four reactors. To ensure that the initial conditions were consistent for each set of experiments they were inoculated with a consistent culture from the inoculum tank (discussed in Chapter 3).

The phosphorus and nitrogen concentrations tested are within the high and low levels found in domestic wastewater according to Metcalf and Eddy (2003).

The light intensities tested were 60 and 150 $\mu E/m^2.s$. The exact depth of where these light intensities would occur in a full scale WSP depends on a number of factors including the solids concentration in the pond water, humic substances and the weather conditions (eg cloud cover). For example, under clear sky at the Ashhurst WSP, New Zealand the two tested light intensities corresponded to approximately 10 cm below the surface and 30 cm below the surface.

The summer and winter sunrise and sunset times for Wellington, New Zealand, were used to establish the timing used for diurnal light cycle. To simulate summer conditions the lights were on for 15 hours and off for 9 hours and for the winter they were on for 9 hours and off for 15 hours.

2.3 Results and discussion

2.3.1 Experimental results

The results from the experiments are shown in Figure 2.1. The conditions tested in each experiment are listed in Table 2.3. The phosphate concentration showed a general decrease over time and in some cases reached zero. The dry weight concentration showed an increase over time and in some experiments levelled off but in others the dry weight concentration was approximately linear and continued to occur even when there were very low levels of phosphate in the medium, for example Experiment M. This will be discussed further in later sections.

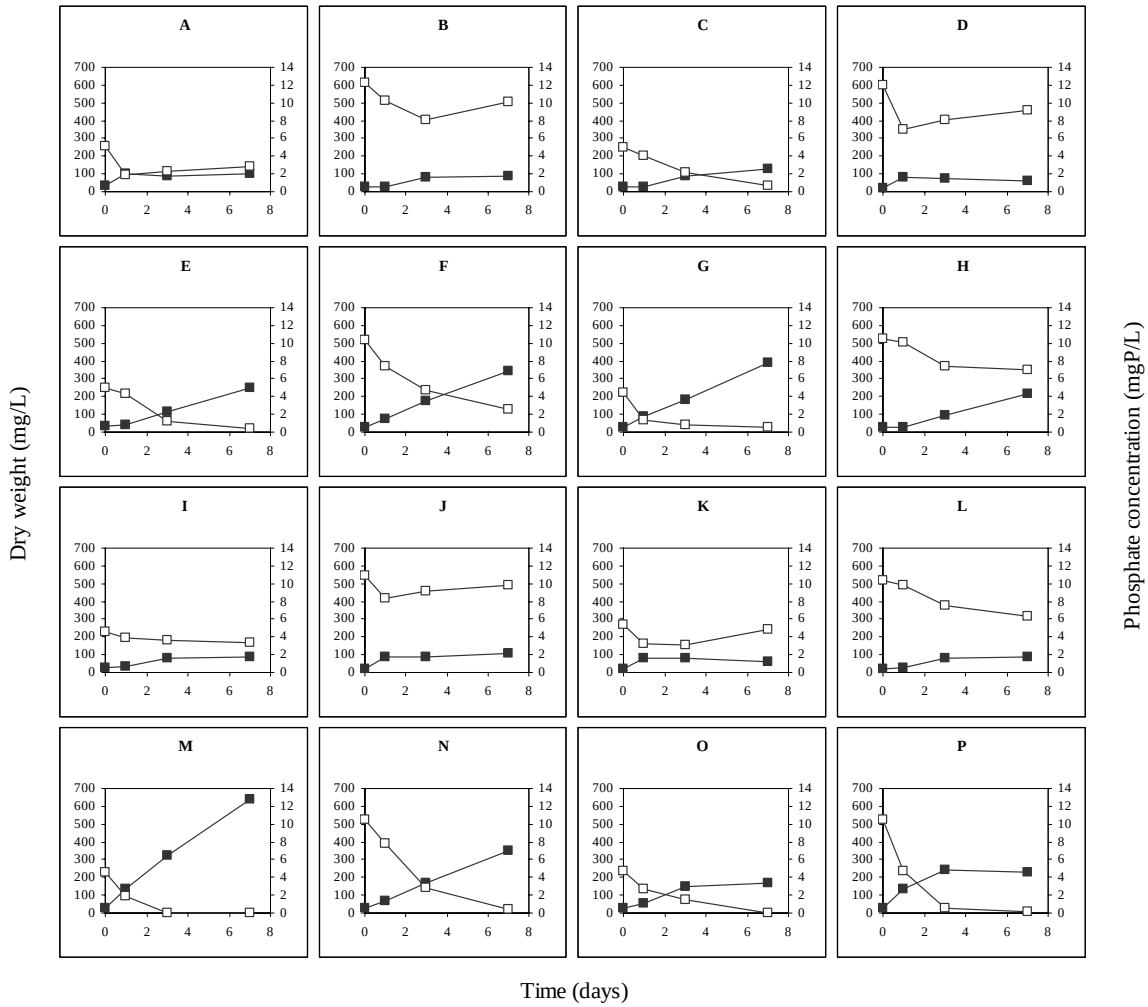

Figure 2.1: Data collected from the factorial experiments for dry weight (■) and phosphate concentration (□)

2.3.2 Statistical analysis

The factors that are considered as having a significant effect are those with at least 95% confidence or a p-value of 0.05 or lower. Table 2.4 shows the p-values for each of the factors with the significant factors shown in bold. The effect of the interactions between factors are also shown in Table 2.4 with a * symbol.

*Table 2.4: p-values from statistical analysis (with significant p-values in bold and interactions shown by a * symbol)*

Variable	Phosphorus removed	Biomass produced [c]
Phosphorus[a]	**0.001**	0.332
Nitrogen[b]	0.288	0.081
Light intensity	**0.000**	**0.000**
Diurnal light cycle	0.755	**0.043**
Temperature	**0.006**	**0.001**
Phosphorus[a] * Nitrogen[b]	0.504	0.577
Phosphorus[a] * Light Intensity	0.267	0.266
Phosphorus[a] * Diurnal	**0.008**	0.902
Phosphorus[a] * Temperature	0.884	0.061
Nitrogen[b] * Light Intensity	0.093	0.093
Nitrogen[b] * Diurnal	0.065	0.068
Nitrogen[b] * Temperature	0.270	0.292
Light Intensity * Diurnal	**0.000**	0.082
Light Intensity * Temperature	0.265	**0.003**
Diurnal * Temperature	0.803	0.494

[a] initial phosphorus concentration in the synthetic wastewater; [b] initial nitrogen concentration in the synthetic wastewater; [c] biomass produced represents the change in dry weight concentration

2.3.2.1 Biomass growth

Table 2.4 shows that biomass growth measured as the change in dry weight was statistically affected by light intensity, diurnal light cycle and temperature. This is not unexpected as the diurnal light cycle and light intensity effect growth as they provide the light required for photosynthesis. The temperature also affects growth as it influences the rate of biological reactions and affects the solubility of carbon dioxide and oxygen. Growth was not affected by phosphorus or nitrogen concentration which indicates that the levels used were not limiting.

2.3.2.2 Phosphorus uptake

Phosphorus uptake was statistically affected by the initial phosphate concentration, light intensity and temperature. Light intensity and temperature are important for algal growth and would therefore also affect phosphorus uptake. Phosphorus uptake is generally stimulated by light as it is an energy dependent reaction and the energy required can be supplied by photosynthesis (Kaplan et al., 1986; Grobbelaar, 2004). An interesting result is that the initial phosphorus concentration affected the amount of phosphorus removed but not the amount of growth. This indicates that there is another biological phosphorus uptake mechanism occurring other than growth, which will be discussed in further sections.

2.3.2.3 Interactions between factors

The statistical analysis also allows the interactions occurring between factors to be investigated. The amount of phosphorus removed was statistically affected by the interaction between phosphorus concentration and diurnal light cycle and between light intensity and diurnal light cycle. The only interaction that affected biomass produced was between light intensity and temperature. This interaction was probably due to the effect that light intensity and temperature is known to have on the photosynthetic rate (Richmond, 1986).

2.3.3 Assimilation for growth versus polyphosphate accumulation

Many of these results were not unexpected; however, what was particularly interesting was that further analysis of the results indicated the occurrence of a biological phosphorus uptake mechanism other than growth. Microalgae contain approximately 1% phosphorus per unit dry weight (Borchardt & Azad, 1968; Kaplan et al., 1986) so the amount of phosphorus uptake due to growth can be estimated.

Figure 2.2 shows the phosphorus that was theoretically removed due to growth and the actual uptake measured for Experiment N. If the phosphorus uptake was only due to growth these two lines would be the same but Figure 2.2 shows there is a large amount of phosphorus uptake that occurred due to another biological mechanism.

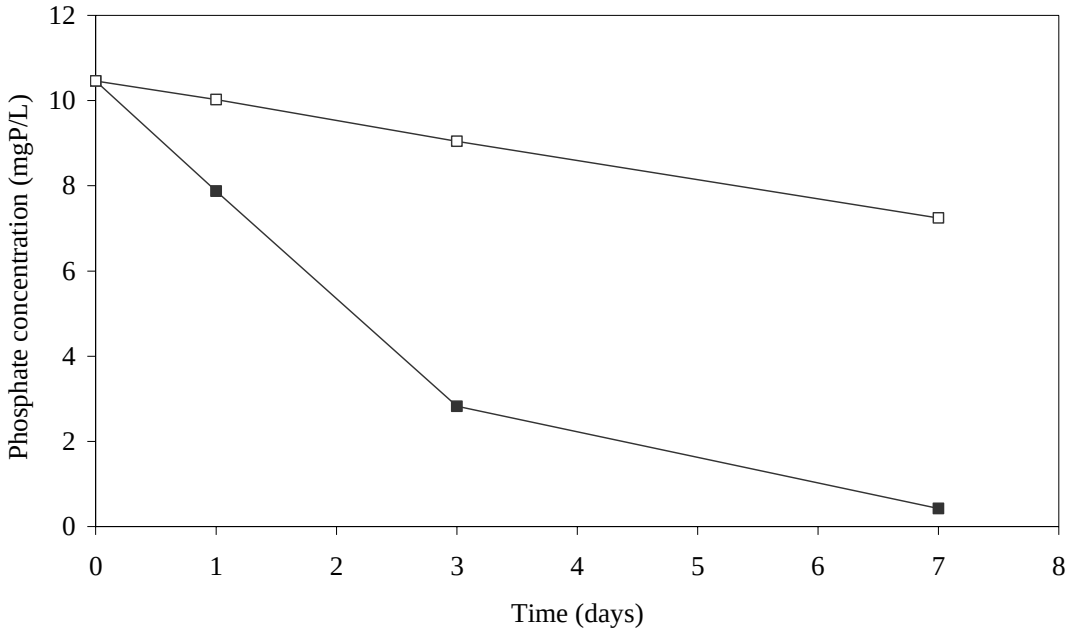

Figure 2.2 Phosphate uptake measured (■) and theoretically predicted uptake for growth (□) for Experiment N.

A biological phosphorus uptake mechanism that could explain the results seen in Figure 2.2 is luxury uptake. Luxury uptake is the storage of phosphorus in the cells as polyphosphate. Further evidence that luxury uptake may have occurred is shown in Figure 2.1. For example in Experiment M growth continued even after there was no phosphate left in the medium, indicating that another source of phosphorus apart from that in the liquid was available to the cells for growth. This would indeed be the case when phosphorus has been stored as polyphosphate within the algal cells.

Another indication that luxury uptake may be a significant mechanism is that the initial phosphate concentration affected the amount of phosphate removed from the wastewater but not growth. At higher phosphate levels there was more phosphate removed but this did not correspond with an increase in growth. This indicates that some of the phosphate taken up by the cells was being stored which could be explained

by luxury uptake. Therefore, further research is warranted to confirm that luxury uptake is occurring and to investigate the factors that influence this mechanism.

2.4 Conclusions

These experiments have shown that the key environmental factors that significantly affect biological phosphorus uptake are the phosphate concentration, light intensity and temperature. Further experiments will focus on these key environmental factors to study the mechanisms involved in more detail.

It was shown that biological phosphorus uptake was not due to biomass growth alone. Growth continued to occur when there was no external phosphate source. Furthermore, at higher phosphate concentrations more phosphate was removed but it did not result in higher growth. This behaviour can be explained by a mechanism known as luxury uptake which may indeed be a key phosphorus uptake mechanism in WSP.

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Chapter 3

Does luxury uptake occur?

Chapter preface

The second objective of this thesis was to determine whether luxury uptake of phosphorus occurs in microalgae under the conditions that typically occur in WSP. It has been hypothesised in Chapter 2 that luxury uptake of phosphorus may be occurring in microalgae cultured under typical conditions present in WSP. This chapter further investigates the biological phosphorus uptake mechanisms occurring and uses three methods to determine whether luxury uptake of phosphorus is indeed occurring. The methods used to investigate the occurrence of luxury uptake were microbiological staining, measuring the percentage phosphorus in the biomass and polyphosphate extraction and quantification. Potential phosphorus uptake by microalgae is also assessed using continuous culture reactors.

This chapter is based on the following publication:

Powell, N., Shilton, A., Pratt, S., & Chisti, Y. (2006). Luxury uptake of phosphorus by microalgae in waste stabilisation ponds. In R. Stuetz & L. Teik-Thye (Eds.), *Young Researchers 2006 (Water and Environment Management Series no. 12)* (pp. 249-256). London: IWA Publishing.

Abstract

Waste stabilisation ponds (WSP) effectively remove organic carbon and pathogens but their capacity to remove phosphorus is generally limited. Some phosphorus can be removed biologically but there has been very little research into the biological mechanisms involved. Organisms remove phosphorus for growth but there are also some microalgae that are capable of storing phosphorus as polyphosphate under certain conditions; a process known as luxury uptake. This chapter investigates potential phosphorus uptake using continuous culture reactors and investigates whether luxury uptake can occur in WSP microalgae. Initially 100% phosphorus uptake was achieved but a change in dominating species from *Scenedesmus* to filamentous microalgae resulted in a decrease in uptake to approximately 60%. Three techniques which have not previously been used for studying phosphorus uptake in WSP were applied. These techniques were staining cells, measurement of the percentage phosphorus in the dry weight and phosphorus fractionation. Staining microalgal cells with a stain specific for polyphosphate granules showed a large number of granules were present in the microalgae. Normally microalgal biomass contains 1% phosphorus per dry weight but the biomass in the reactors contained 1.3% and 2.0% for filamentous and *Scenedesmus* respectively. The polyphosphate fractions were also extracted from the biomass and showed a large amount of polyphosphate present. All these techniques used to detect luxury uptake implied that luxury uptake was indeed occurring in these reactors.

3.1 Introduction

Biological phosphorus removal occurs in WSP as phosphorus is a part of many biological compounds needed for organism growth. However, another biological phosphorus removal mechanism that could occur in ponds is luxury uptake by microalgae. This is the accumulation of phosphorus in microalgae in the form of polyphosphate. It has been found that in natural systems such as lakes some microalgae store phosphorus that can then be used as an internal source of phosphorus when the external phosphorus becomes limiting (Kuhl, 1974). It is possible that luxury uptake could occur in microalgae under WSP as hypothesised in Chapter 2; however, this mechanism has not previously been investigated in microalgae under the environmental conditions that occur in WSP.

In this chapter, three techniques are used to investigate whether luxury uptake of phosphorus can occur in microalgae under the conditions typically found in WSP. Potential phosphorus uptake by the microalgae is also assessed

3.2 Methods

3.2.1 Reactor setup

Two continuous reactors were set up to simulate the algal band of a WSP in the laboratory. The first (Reactor 1) was inoculated with wastewater from a WSP in Ashhurst, New Zealand. A second continuous reactor (Reactor 2) was then started using Reactor 1 as an inoculum.

The reactors had a volume of twelve litres and were fed with synthetic wastewater according to Davis and Wilcomb (1967). The composition of the synthetic wastewater is given in Chapter 2 (Tables 2.1 and 2.2). This synthetic wastewater contained chelating chemicals to prevent precipitation so that the biological mechanisms can be studied. The retention time in the reactors was ten days.

Daylight fluorescent lights (Philips, 36W) were used to simulate day and night (15 hours light and 9 hours dark) and the light intensity at the surface was 150 $\mu E/m^2.s$. The light intensity was monitored using an Irradiance Sensor (QSL-2101; Biospherical Instruments, San Diego, CA, USA). Because of the changing light intensity simulating day and night a true steady-state does not occur, instead a pseudo steady-state was reached. The reactor was in a constant temperature room at 25 °C and slowly stirred with a mechanical stirrer. The sides of the reactor were covered to prevent growth on the walls. A diagram of a reactor is shown in Figure 3.1.

The phosphate in the feed and phosphate in the effluent were monitored using ion chromatography (Dionex ICS-2000; Dionex Corporation, Sunnyvale, CA, USA) in duplicate as described in Chapter 2. The biomass concentration was measured as dry weight according to Lee and Shen (2004). The microbial species present in the reactors were also regularly examined under the microscope.

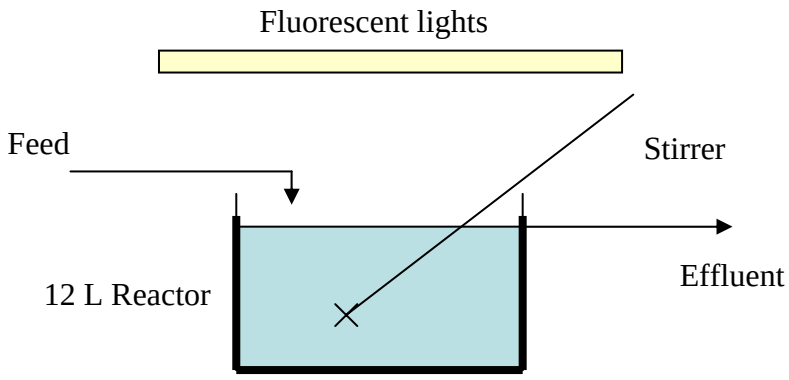

Figure 3.1: Diagram of reactor

3.2.2 Techniques for investigating luxury uptake

Luxury uptake can be investigated in a number of ways. The cells can be stained for polyphosphate granules and then examined under the microscope, the percentage phosphorus in the cells can be measured, or the polyphosphate can be extracted from the cells and measured.

All three techniques were applied in this study. These methods have not previously been applied to studying phosphorus uptake in WSP.

3.2.2.1 Polyphosphate granule stain

Luxury uptake can be confirmed by using a stain specific for polyphosphate granules in microalgae. Bolier et al. (1992) developed a staining technique which stains the polyphosphate granules dark brown. The cells are then examined under the microscope and the presence of polyphosphate granules can be quickly confirmed.

3.2.2.2 Percentage phosphorus in biomass

Microalgae require phosphorus as a nutrient for growth. The amount of phosphorus utilised for growth can be calculated as microalgae contain approximately 1% phosphorus by dry weight (Borchardt & Azad, 1968; Kaplan et al., 1986). The amount of phosphorus in the biomass can be measured by determining the total phosphorus

using the nitric acid and sulphuric acid digestion and the ascorbic acid method according to standard methods (APHA et al., 1995) and by measuring the dry weight. This value can then be compared to the 1% phosphorus per dry weight for normal growth and any value higher that this indicates that luxury uptake is occurring.

3.2.2.3 Polyphosphate extraction

To confirm the presence and quantify the amount of polyphosphate in the microalgae a method of fractionating the different types of phosphorus in the algal biomass was used. This method was developed by Aitchison & Butt (1973) and Kanai et al., (1965) to study phosphorus metabolism in microalgae. The samples were centrifuged to form an algal pellet which was then washed using deionised water. This was then centrifuged and the liquid was discarded. The acid-soluble polyphosphate was then extracted using 5mL of 10% trichloroacetic acid at 5°C for 5 minutes. This extraction was conducted a second time and the extracts were pooled after centrifugation. The next extraction step was to remove the lipid phosphate which was conducted by extracting twice with 5mL of ethanol for 5 minutes and once with 5mL of ethanol:ether (3:1). The acid-insoluble polyphosphate was then extracted using 10mL of potassium hydroxide for 10 minutes. This was then repeated using 2mL of potassium hydroxide. The polyphosphate fractions were then analysed for total phosphorus using the nitric acid and sulphuric acid digestion and the ascorbic acid method according to standard methods (APHA et al., 1995).

3.3 Results and discussion

3.3.1 Phosphate in effluent

Initially for Reactor 1 there was no detectable phosphate in the effluent as shown in Figure 3.2. This period of 100% phosphorus uptake was sustained for the first 4–5 months after which phosphate began to appear in the effluent. Towards the end of the experiment the effluent phosphate concentration fluctuated but was approximately 2 mgP/L (57% uptake). Phosphorus removal in WSP systems is usually between 15–50% (Picot et al., 1992; Racault et al., 1995; Garcia et al., 2000) so this level of uptake would not be untypical however, the initial 100% phosphorus uptake is exceptional.

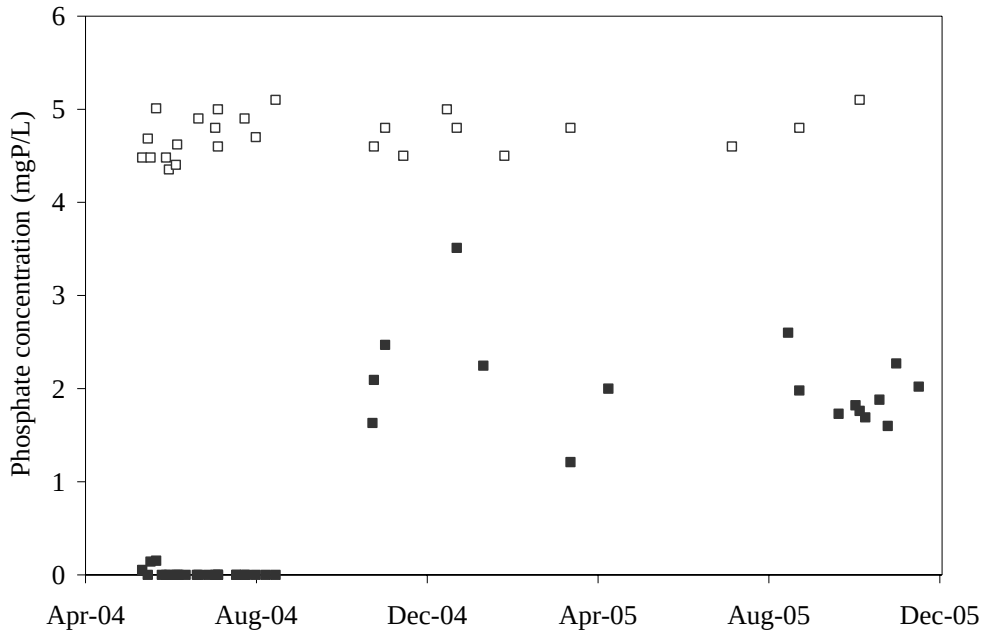

Figure 3.2: Phosphate in the feed (□) and effluent (■) of Reactor 1

The phosphorus removal from the synthetic wastewater is not due to precipitation as chelating chemicals were added to prevent precipitation occurring at the high pH levels achieved and high phosphorus uptake was still able to be obtained. This means that the phosphorus has been removed due to growth alone or due to a combination of growth and luxury uptake of phosphorus.

3.3.2 Biomass concentration

The biomass concentration for Reactor 1 measured as dry weight is shown in Figure 3.3. Initially Reactor 1 reached a pseudo steady-state dry weight concentration of approximately 260 mg/L. At that stage the dominating species was *Scenedesmus*. There was then a slow change in species from *Scenedesmus* to filamentous green algae. This caused an increase in the dry weight concentration to approximately 670 mg/L. The cause for the species change is unknown but it may be due to the filamentous algae reaching a significant population by growing on the walls of the reactor.

When the species began to change a second continuous reactor (seeded from the original reactor) was started. The operating conditions were the same as the original reactor. It was observed that the second reactor was dominated with *Scenedesmus* with a

small population of the filamentous species. This second reactor was then used for measurements to compare the performance of the two dominating species.

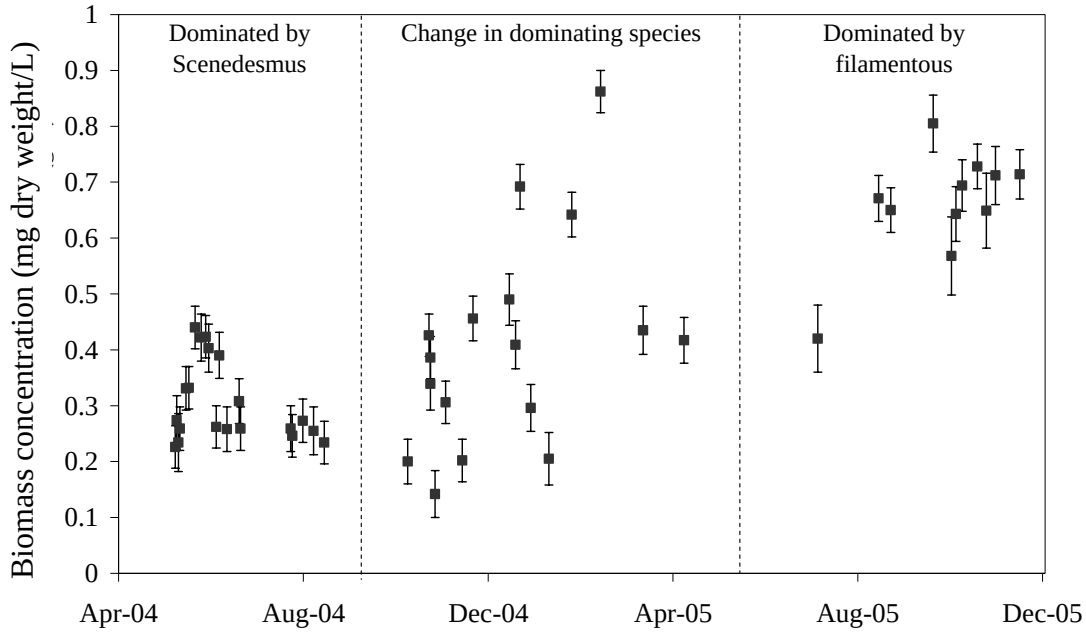

Figure 3.3: Biomass concentration and dominating microalgae in Reactor 1

The dry weight concentration in the laboratory reactor is much higher than the suspended solids found in a WSP. WSP generally contain an algal biomass concentration of 10–100 mg/L and high rate algal ponds (HRAP) can get up to 1500 mg/L (Abeliovich, 1986). In a WSP only the top 20–30 cm of the pond contains enough light for microalgal growth to occur but the ponds are generally 1–1.5 m deep so the algae that grow in the top algal band are then diluted by the rest of the pond. The laboratory reactors were only 20 cm deep so there were no dilution effects. If the same dilution occurred for the laboratory experiments the dry weight concentration would have been 52 mg/L and 134 mg/L for *Scenedesmus* and the filamentous microalgae respectively. Similar dilution effects have been reported previously when comparing the algal concentration in samples taken from the entire water column compared to the concentration within the algal band (Pearson & Konig, 1986).

3.3.3 Confirmation of luxury uptake using staining

Figure 3.4 shows the large number of polyphosphate granules stained dark brown in the microalgal cells. This observation has been reported in a previous paper (Powell et al., 2005). This confirms that luxury uptake is occurring in these microalgae.

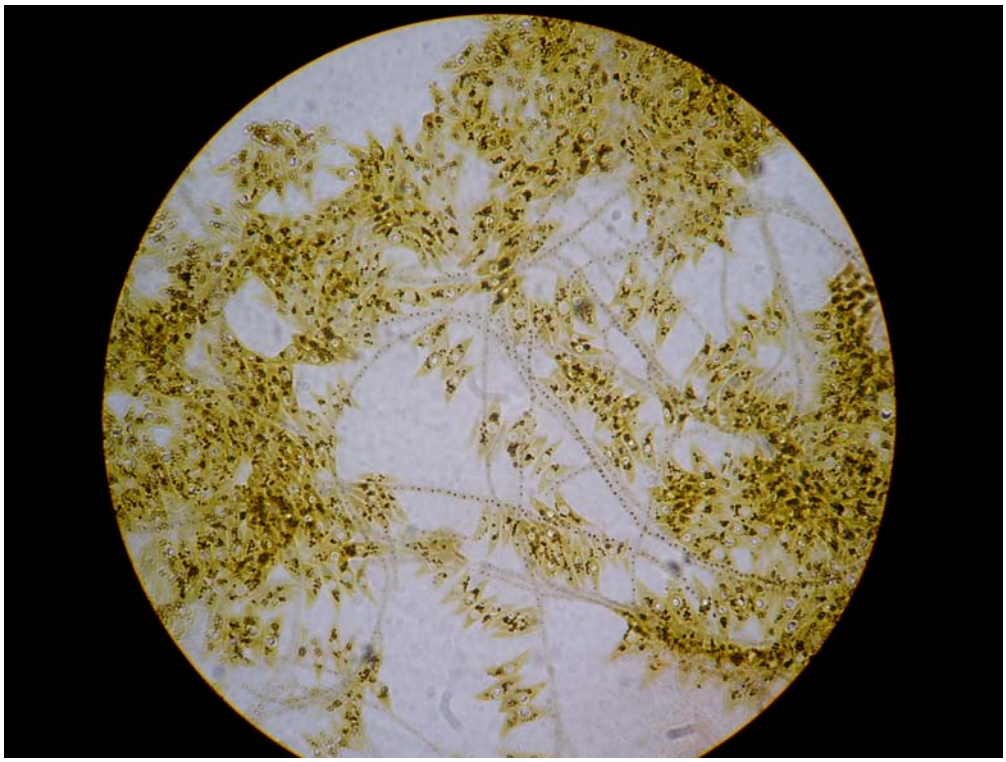

Figure 3.4: Microalgal cells stained for polyphosphate granules (Powell et al., 2005)

3.3.4 Measurement of percentage phosphorus in biomass

Algae contain approximately 1% phosphorus per dry weight for normal metabolism (Borchardt & Azad, 1968; Kaplan et al., 1986). Measurement of the total phosphorus of the biomass and the dry weight show that the culture dominated by filamentous algae (Reactor 1) contained an average of 1.29% phosphorus per dry weight and the culture dominated by *Scenedesmus* (Reactor 2) contained an average of 2.02% phosphorus per dry weight. The result indicates that phosphorus was being stored beyond that required for biomass growth which would be expected if luxury uptake was occurring.

3.3.5 Confirmation of luxury uptake using phosphorus fractionation

The amount of polyphosphate in the microalgae can be determined by chemical fractionation. This method quantifies the fractions as acid-soluble polyphosphate (ASP), acid-insoluble polyphosphate (AISP) and other forms of cellular phosphorus. Figure 3.5

shows the phosphorus fractions for both filamentous microalgae (Reactor 1) and *Scenedesmus* (Reactor 2). The polyphosphate results are reported per unit dry weight to correct for any differences in biomass concentration between the reactors. Even when filamentous algae dominated which resulted in reduced phosphorus uptake, the biomass contained a large amount of polyphosphate, which is indicative of luxury uptake.

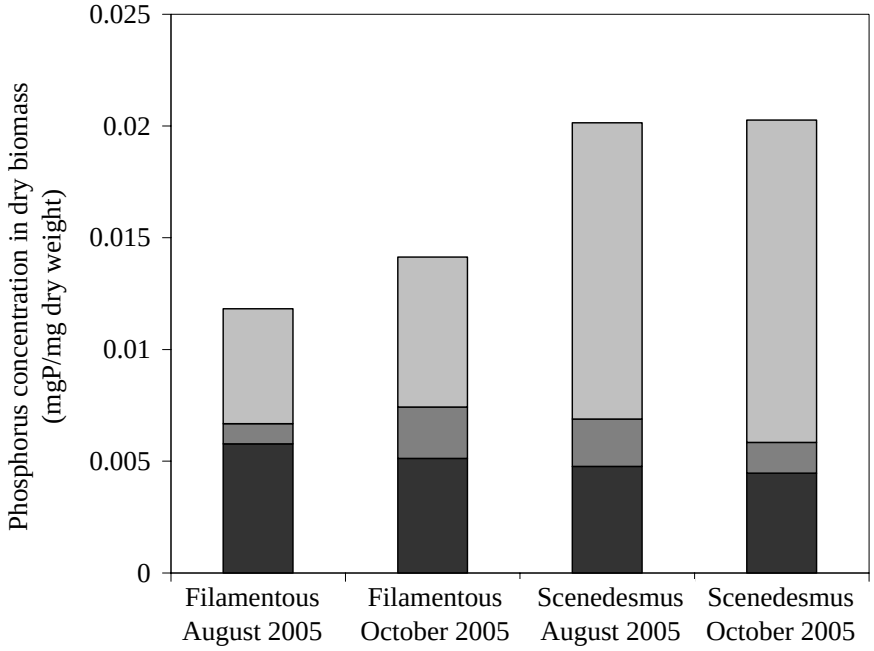

Figure 3.5: Phosphorus fractions in two microalgal species. Acid-soluble polyphosphate shown in black, acid-insoluble polyphosphate shown in medium grey and other forms of cellular phosphorus shown in light grey.

Figure 3.5 also shows that *Scenedesmus* contains more phosphorus than the filamentous species which may explain why there was better phosphorus uptake when *Scenedesmus* dominated. The amount of polyphosphate in the two species is similar however the other cellular phosphorus fraction shows that the culture dominated by *Scenedesmus* contained more phosphorus in some other form. Other cellular compounds that contain phosphorus include phospholipids, nucleotides, nucleic acids and proteins.

3.4 Conclusions

The experimental results show that under the conditions tested the WSP microalgae could achieve 100% phosphorus uptake. However, after approximately five months the phosphorus uptake decreased. It is possible that the change in phosphorus uptake is due to the change in algal species from *Scenedesmus* to filamentous microalgae. This is because the drop in performance occurred at the same time as the change in species and the results show that the culture dominated by S*cenedesmus* contained more phosphorus per dry weight than the filamentous species.

All three techniques for detecting luxury uptake indicate that polyphosphate is being stored by microalgae under the conditions that occur in the WSP. These methods have not previously been applied to studying phosphorus uptake in WSP. The staining technique shows a large number of granules, the percentage phosphorus in the biomass is above 1% and the phosphorus fractions show that there is a significant amount of polyphosphate in the biomass.

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Chapter 4

Factors influencing luxury uptake

Chapter preface

The screening experiments reported in Chapter 2 showed that biological phosphorus uptake is influenced by the phosphate concentration in the synthetic wastewater, light intensity and temperature. Furthermore, Chapter 3 has now shown that luxury uptake of phosphorus occurs in microalgae grown under the conditions that typically occur in WSP. This chapter uses continuous culture reactors to build on these findings and begin to explore the third objective of the thesis which is to determine how key environmental factors influence the luxury uptake mechanism in microalgae under WSP conditions.

This chapter is based on the following publication:

Powell, N., Shilton, A., Pratt, S., & Chisti, Y. (2008). Factors influencing luxury uptake of phosphorus by microalgae in waste stabilization ponds. *Environmental Science and Technology, 42(16), 5958-5962*.

Abstract

Phosphorus removal in waste stabilisation ponds (WSP) is highly variable but the reasons for this are not well understood. Luxury uptake of phosphorus by microalgae has been studied in natural systems such as lakes but not under the conditions found in WSP. This work reports on the effects of phosphate concentration, light intensity and temperature on luxury uptake of phosphorus by WSP microalgae in continuous culture reactors. Increasing temperature had a statistically significant 'positive effect' on intracellular acid-insoluble polyphosphate (AISP) concentration. It is likely that elevated temperature increased the rate of polyphosphate accumulation, but as the microalgae were not starved of phosphate the stored AISP was not consumed. Increasing light intensity had no effect on AISP, but had a 'negative effect' on the acid-soluble polyphosphate (ASP). A possible explanation for this is that the faster growth rate at high light intensity results in this form of polyphosphate being consumed by the cells for synthesis of cellular constituents at a rate that exceeds replenishment. The findings of this chapter suggest that by manipulating the factors which influence luxury uptake there is the potential to optimise WSP for biological phosphorus uptake.

4.1 Introduction

Growth of microalgae consumes phosphorus as an essential element needed for cellular constituents such as phospholipids, nucleotides, and nucleic acids (Miyachi et al., 1964). Another biological phosphorus uptake mechanism that has been shown to occur under conditions relevant to WSP is luxury uptake (as discussed in Chapter 3). Luxury uptake is the storage of phosphorus within the biomass in the form of polyphosphate. Polyphosphate can be present in microalgae as either acid-soluble polyphosphate (ASP) or acid-insoluble polyphosphate (AISP). ASP is thought to be actively involved in metabolism while AISP is stored for when the external phosphate concentration becomes limiting (Miyachi et al., 1964).

Screening experiments have identified the key environmental factors that affect biological phosphorus uptake in WSP microalgae (Chapter 2). These factors are the phosphate concentration, light intensity and temperature. While the experiments conducted in Chapter 2 identified the factors that influenced biological phosphate

uptake, they did not establish how these factors influence luxury uptake. This chapter uses continuous culture reactors operated under controlled conditions to assess the impact of these key environmental factors on luxury uptake.

4.2 Materials and methods

4.2.1 Experimental setup

The effect of phosphorus concentration, light intensity and temperature on the percentage phosphorus and polyphosphate in the biomass were assessed using a full factorial experimental design requiring a total of eight reactors. The experimental matrix is shown in Table 4.1. Two experimental runs were undertaken each with four reactors. At the start of the experiments the reactors were inoculated with broth from a continuous culture inoculum reactor dominated by *Scenedesmus* sp. (Reactor 2 in Chapter 3). Details of this continuous culture inoculum reactor have been reported in Chapter 3.

Table 4.1: Experimental design used for the continuous reactors

Reactor	**Phosphate (mgP/L)**	**Light intensity ($\mu E/m^2 \cdot s$)**	**Temperature (°C)**
A	5	60	15
B	15	60	15
C	5	150	15
D	15	150	15
E	5	60	25
F	15	60	25
G	5	150	25
H	15	150	25

Continuous culture reactors (Figure 4.1) were used for all experiments. The reactors had a volume of three litres and a hydraulic retention time of ten days. Culture depth in the reactors was approximately 70 mm. The rectangular reactor vessels were 170 mm wide and 250 mm long. The reactors were fed with synthetic wastewater according to Davis and Wilcomb (1967). The composition of the synthetic wastewater is given in Chapter 2 (Tables 2.1 and 2.2). This synthetic wastewater contained chelating agents to prevent phosphorus precipitation and enable the research to focus on the biological phosphorus mechanisms.

The reactors were mixed using magnetic stirrers (50 mm stirrer bars). Lighting was provided by fluorescent daylight lamps (Philips, 36W) set on timers to simulate day and night (15 hours light and 9 hours dark). The light intensity was monitored at the surface of the reactors using a PAR (photosynthetically active radiation) irradiance sensor (QSL-2101; Biospherical Instruments, San Diego, CA, USA). All experiments were conducted in a controlled temperature room.

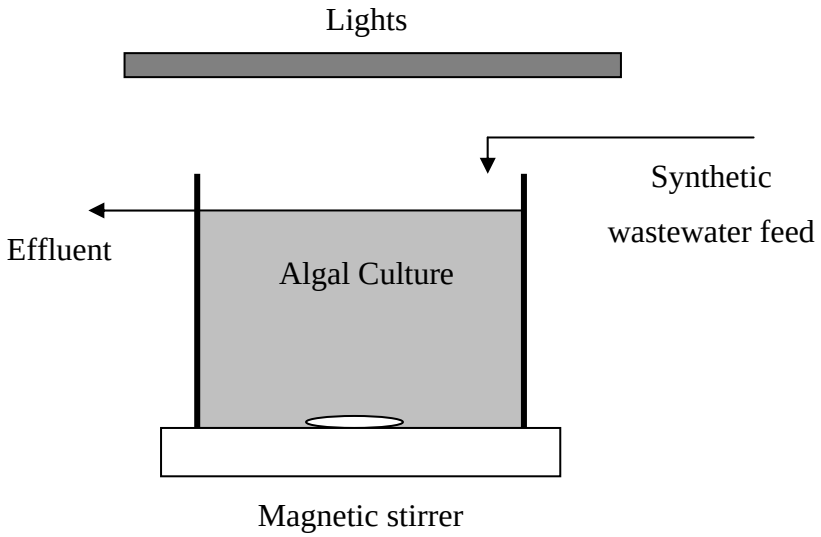

Figure 4.1: Continuous culture reactor

4.2.2 Analytical methods

The phosphate and biomass concentration of the reactor effluent was monitored to determine when quasi steady-state had been reached. A quasi steady-state was reached due to the changing day/night cycle. Quasi steady-state was defined to occur when three

consecutive measurements were within ±10%. The phosphate concentration was measured using ion chromatography (Dionex ICS-2000; Dionex Corporation, Sunnyvale, CA, USA) as described in Chapter 2. The biomass was recovered on a 0.45 µm membrane filter and measured as dry weight (Lee & Shen, 2004). All samples were analysed in duplicate.

Once a quasi steady-state had been reached in the continuous culture reactors, the analysis of the different forms of phosphorus was undertaken. The forms of phosphorus analysed included the phosphate in the biomass-free liquid, the total phosphorus (TP) in the biomass-free liquid and the TP of the biomass. The TP was measured according to standard methods (APHA et al., 1995) using the nitric acid and sulphuric acid digestion and analysis using the ascorbic acid method.

The polyphosphate was analysed using the chemical extraction technique described by Aitchison & Butt (1973) and Kanai et al. (1965). This methods involves a series of extractions using trichloroacetic acid, ethanol, ethanol: ether (3:1 by volume) and potassium hydroxide.

4.2.2 Statistical analysis

The results were analysed using the statistical software MINITAB (Minitab Inc., State College, PA, USA). Factors are reported as significant at either 95% confidence (p-value less than or equal to 0.05) or 90% confidence (p-value between 0.05 and 0.10).

4.3 Results and discussion

Results of the continuous culture experiments are reported here as the percentage phosphorus and the polyphosphate content per unit dry weight. Implications of these measurements for phosphorus removal in WSP are then discussed.

4.3.1 Percentage phosphorus in biomass

The average percentage phosphorus per unit dry weight for each reactor is shown in a cube plot in Figure 4.2. A cube plot was used to enable the effects of each of the factors tested to be identified. The average percentage phosphorus per dry weight was 1.27% with a maximum of 3.16% and a minimum of 0.41%. In the absence of luxury uptake, microalgae typically contain 1% phosphorus per dry weight (Borchardt & Azad, 1968; Kaplan et al., 1986). These results suggest that luxury uptake was occurring (Borchardt & Azad, 1968). The highest percentage phosphorus occurred (Figure 4.2) at high temperature (25 °C) and low light intensity (60 µE/m^2·s). The lowest percentage phosphorus occurred at low temperature (15 °C) and low phosphorus concentration (5 mg/L). To determine which effects are significant, a statistical analysis is required.

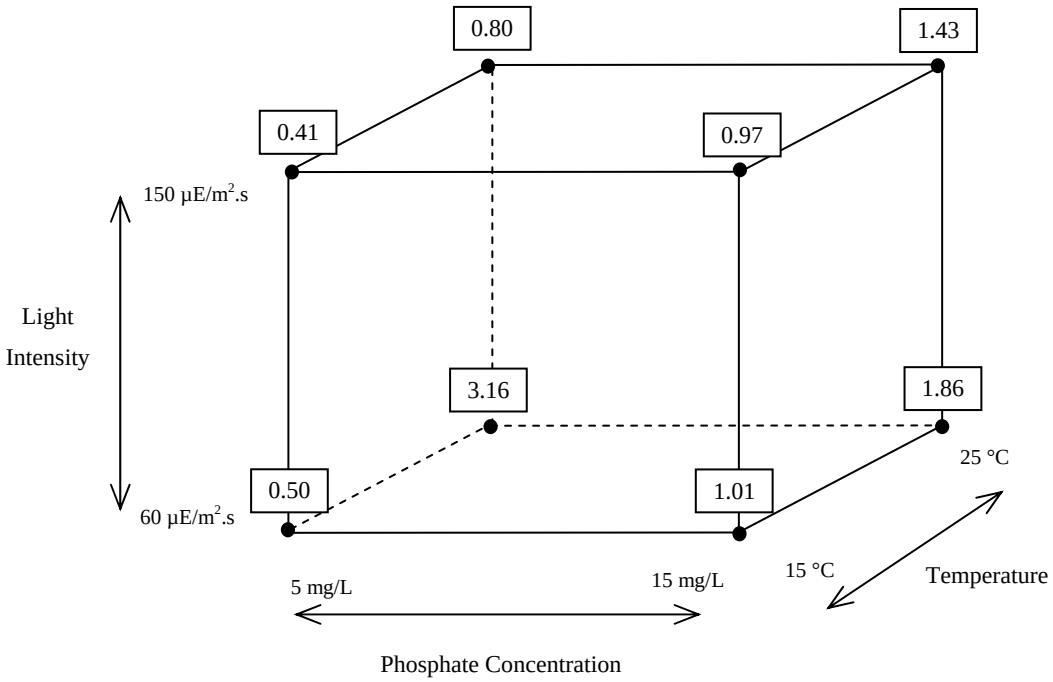

Figure 4.2: Percentage phosphorus per unit dry weight at quasi steady-state

Table 4.2 shows the results of the statistical analysis and reports the p-values for the factors. Light intensity was found to have a negative effect on the phosphorus content of the biomass (Table 4.2) meaning that the reactors operated at low light intensity had a higher amount of phosphorus in their biomass compared with those at higher light intensity. This was unexpected as phosphorus uptake is known to be an energy requiring process (Sato & Murata, 1980; Kaplan et al., 1986; Martinez et al., 1999; Hessen et al., 2002) and microalgae receive their energy via photosynthesis that is generally enhanced

with increasing intensity of light. Nonetheless, the observed negative effect of light intensity on phosphorus content was consistent with other published reports on the phosphorus content of microalgae in natural systems. For example, Hessen et al. (2002) reported that the phosphorus-to-carbon ratio in green microalga *Selenastrum* increased with reducing light intensity. Similar results were observed by Martinez et al. (1999) in studies of phosphorus metabolism of *Scenedesmus*. Possible reasons for this negative effect will be discussed in later sections of this chapter.

Table 4.2: Statistical analysis of the percentage phosphorus per unit dry weight

Variable	p-value	Significance	Effect
Phosphate[a]	0.634	Not significant	N/A[b]
Light Intensity	0.006	95% confidence	Negative
Temperature	0.000	95% confidence	Positive
Phosphate[a] * Light Intensity	0.036	95% confidence	Positive
Phosphate[a] * Temperature	0.059	90% confidence	Negative
Light Intensity * Temperature	0.009	95% confidence	Negative

[a] Phosphate concentration in the synthetic wastewater; [b] Not applicable.

The temperature had a positive effect on the percentage phosphorus per unit dry weight (Table 4.2). Temperature is known to not only affect the rate of biological reactions but also the cell composition. Temperature has been reported to affect the fatty acid composition (Sato & Murata, 1980), protein concentration (Payer et al., 1980) and the nitrogen-to-carbon ratio (Goldman, 1977).

The effect of phosphate concentration in the synthetic wastewater on the percentage phosphorus per unit dry weight was not statistically significant (Table 4.2). In other studies (Aitchison & Butt, 1973), the phosphate concentration has been reported to profoundly affect the phosphorus content of microalgal biomass, but this appears to apply to natural environments in which cells are starved of phosphate for periods of

time before being re-exposed. This situation does not generally occur in WSP as phosphate concentration typically exceeds the growth limiting level. These results (Table 4.2) suggest that at the levels tested the phosphate concentration does not have a direct effect on the percentage phosphorus in the microalgae.

Interactive effects of the factors were tested (Table 4.2). An interaction occurs when the effect of a variable is also dependent on another variable. Significant interactions occurred between phosphate concentration and light intensity and also between light intensity and temperature (Table 4.2). The interaction between phosphate concentration and temperature was significant at 90% confidence (Table 4.2). These results show that in the ranges examined the phosphate concentration alone does not significantly affect the percentage phosphorus per unit dry weight, but it is an important variable because of its interactions with both light intensity and temperature.

These results reveal that in continuous culture the principal factors that affect the phosphorus content of WSP microalgae are the light intensity and temperature. The phosphate concentration does not have any direct affect in the 5 to 15 mg/L range, but it is an important variable due to the interactions with the other factors. During luxury uptake, microalgae accumulate phosphorus as polyphosphate. Therefore, to confirm that luxury uptake is occurring direct measurement of polyphosphate in the cells is necessary, as discussed next.

4.3.2 Polyphosphate in biomass

Once a quasi steady-state had been reached, polyphosphate was measured in the biomass samples from continuous culture reactors. To correct for any differences in biomass concentration between the reactors, the polyphosphate is reported as the quantity present per unit of dry weight. The acid-soluble polyphosphate (ASP) and acid-insoluble polyphosphate (AISP) for each reactor is shown in Figure 4.3. Most of the polyphosphate is in the form of ASP (Figure 4.3) which is actively involved in metabolism (Kuhl, 1962).

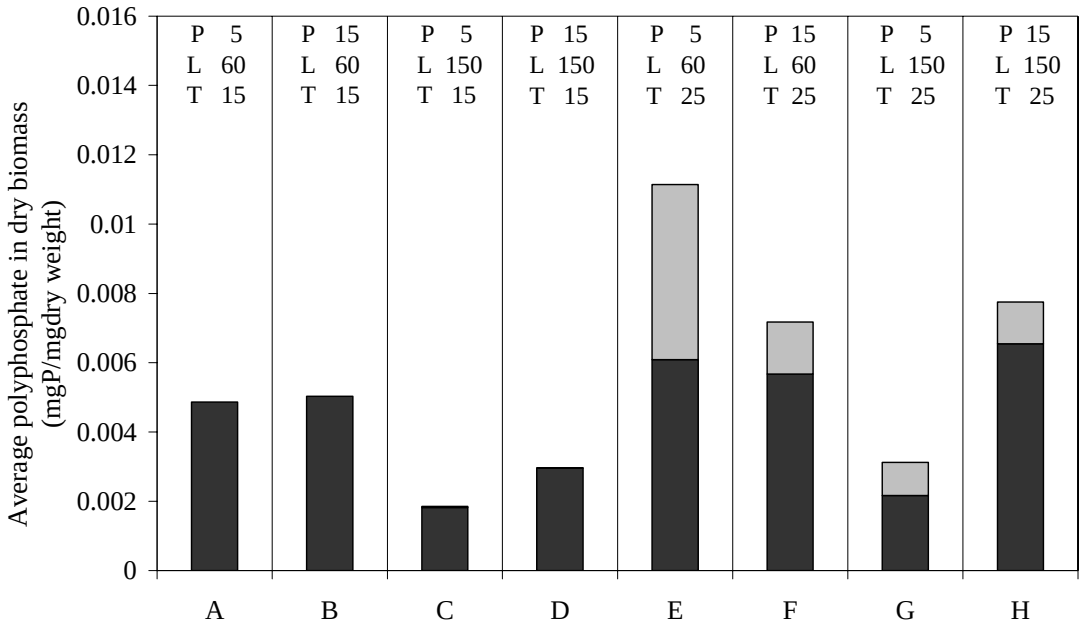

Figure 4.3: Polyphosphate fractions for the eight continuous culture reactors. The phosphate concentration (P), light intensity (L) and temperature (T) for each experiment indicated at top each bar. The black bars represent acid-soluble polyphosphate per unit dry weight. Grey bars denote acid-insoluble polyphosphate per unit dry weight.

Table 4.3 shows the p-values along with the effect of each variable on the ASP and AISP. The phosphate concentration in the synthetic wastewater had no significant affect on the amount of polyphosphate per unit dry weight (Table 4.3). An increase in temperature from 15 °C to 25 °C increased the amount of AISP per unit dry weight but had no significant effect on the ASP (Table 4.3). Barely any AISP was present in the low temperature experiments (Figure 4.3).

According to Miyachi and co-workers (Miyachi & Miyachi, 1961; Miyachi & Tamiya, 1961; Miyachi et al., 1964), AISP is stored in microalgal biomass for use when the external phosphate level becomes limiting for growth. In contrast, ASP is mostly used for the synthesis of cellular constituents under normal conditions. Temperature is known to affect the rate of biological reactions therefore it is likely that higher temperature resulted in an increase in polyphosphate accumulation. However, because the cells were not starved of phosphate the AISP was not consumed which explains the results in Figure 4.3.

Table 4.3: Statistical analysis of the polyphosphate content per unit dry weight

	Acid-soluble polyphosphate			Acid-insoluble polyphosphate		
Variable	**p-value**	**Significance**	**Effect [a]**	**p-value**	**Significance**	**Effect [a]**
Phosphorus	0.176	Not significant	N/A	0.329	Not significant	N/A
Light Intensity	0.043	95% confidence	Negative	0.211	Not significant	N/A
Temperature	0.141	Not significant	N/A	0.022	95% confidence	Positive

[a] N/A: Not applicable.

Light intensity only affected the amount of ASP (Table 4.3). ASP is used by microalgal cells for producing DNA and phosphoprotein (Miyachi et al., 1964). Therefore, the rate of growth and the amount of ASP in the cells are linked. The growth rate is expected to be higher at 150 $\mu E/m^2 \cdot s$ than at 60 $\mu E/m^2 \cdot s$ and, therefore, more ASP is likely consumed in metabolism at the higher light level resulting in a lower concentration in the biomass. This is because the observed amount of polyphosphate per unit dry weight is a consequence of the combined effects of phosphorus accumulation and consumption for metabolism.

The results from experiment G (phosphate concentration 5 mg/L, light intensity 150 $\mu E/m^2 \cdot s$, temperature 25 °C) can also be compared to the continuous reactor reported in Chapter 3 that was dominated by *Scenedesmus*. It is interesting to note that experiment G contained a lower percentage phosphorus and polyphosphate concentration (Figures 4.2 and 4.3) than the reactor in Chapter 3 (Figure 3.5). While the light intensity at the surface of these reactors was the same for both experiments the depth of the reactors was different which would result in the microalgae being exposed to a different light cycle. The reactors used in this chapter had a culture depth of 70 mm while the reactors in Chapter 3 had a culture depth of 200 mm. This would mean that the microalgae in reactor G were exposed to light more frequently which resulted in less polyphosphate being stored. This agrees with the finding that light intensity has a negative effect on polyphosphate content of the microalgae (Table 4.3).

In view of these observations, a better understanding of luxury uptake requires analysis of not only the mass fraction of total phosphorus in the biomass, but also the proportion present as ASP and AISP. It is interesting to note that even at 1% phosphorus which indicates normal metabolism, some polyphosphate is present (Figure 4.2 and 4.3). This shows that the presence of polyphosphate does not necessarily mean that luxury uptake is occurring in the biomass. The percentage phosphorus is useful as it gives an indication of how much phosphorus accumulation has occurred and the polyphosphate extraction shows whether the accumulation is polyphosphate.

4.3.3 Implications for phosphorus removal in WSP

These findings may help explain at least some of the seasonal variation in phosphorus removal that has been documented in WSP. For example, Picot et al., (1992) reported that in a full scale WSP phosphate removal varied between 15% in the winter and 30% in the summer.

Luxury uptake has not previously been identified as a significant phosphorus uptake mechanism in WSP. However, understanding the luxury uptake mechanism could potentially lead to the development of a new technique for biological phosphorus uptake via microalgae. The work presented in this chapter clearly shows that investigating the effects of light intensity and ways to manipulate this variable are important for improving biological phosphorus uptake in WSP. Light intensity varies seasonally and also decreases exponentially throughout the depth of the pond according to Beer's Law. Therefore factors such as the amount of vertical mixing might have important implications for phosphorous uptake. Vertical mixing in WSP occurs naturally due to inlet momentum, action of wind, thermal convection and release of biogas from the anaerobic sludge layer. The degree of vertical mixing determines the frequency of microalgal movement between the relatively better illuminated surface layer and the poorly illuminated interior of the WSP. Phosphate uptake may also be influenced by the predominant algal species in the WSP. For example, motile algae have the ability to move within the pond depth in response to the light intensity.

Biological phosphorus uptake by microalgae is dependent on both the microalgal biomass concentration and the amount of phosphorus that can be accumulated in the

biomass. Figure 4.4 shows the potential biological phosphorus removal from wastewater containing a phosphate concentration of 10 mg/L. The percent phosphorus removal in Figure 4.4 has been calculated for a range of concentrations of microalgal biomass which reflects the typical range found in WSP. The percentage phosphorus levels of 0.4% and 3.2% were selected as these are the minimum and maximum values found in this study (Figure 4.2).

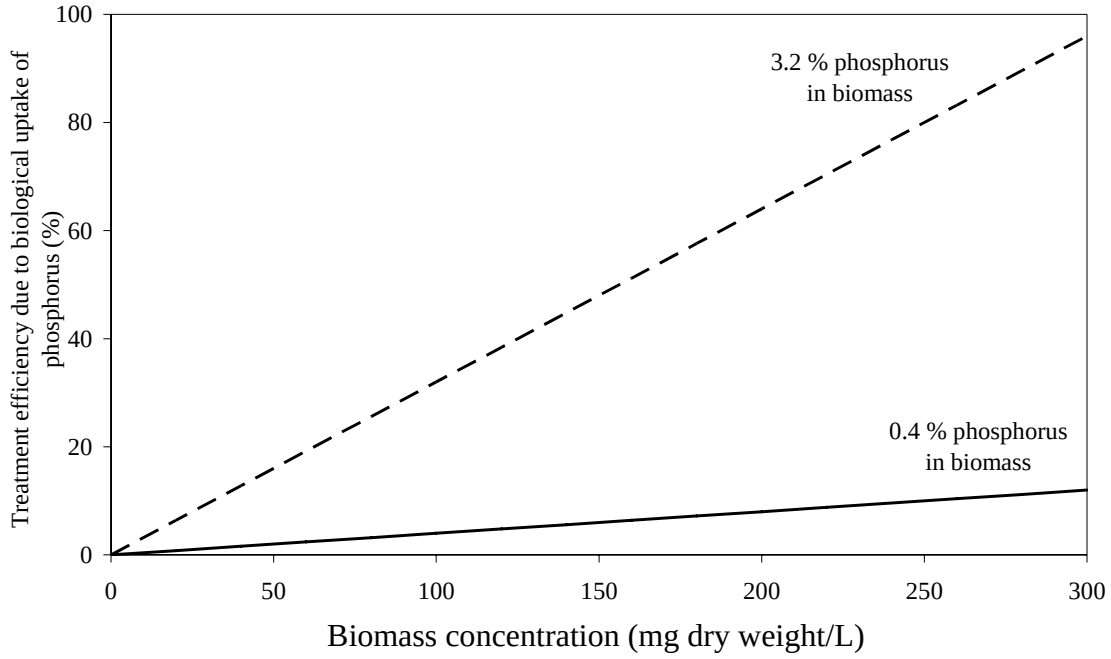

Figure 4.4: Potential biological phosphorus removal in WSP for wastewater with an initial phosphate concentration of 10 mg/L

To achieve this phosphorus removal the microalgae need to be harvested from the WSP. There are a number of established technologies that could effectively remove the algae including dissolved air floatation, microfiltration, sand filtration and sedimentation (Middlebrooks et al., 2005).

Clearly, Figure 4.4 reveals a tremendous potential for biological phosphorus removal in WSP if operation could be optimised to enable maximal phosphorus uptake by the microalgae. As shown in Figure 4.4 to maximise biological phosphorus uptake the biomass concentration and the amount of phosphorus in the biomass need to be maximised.

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Chapter 5

Dynamics of the luxury uptake mechanism

Chapter preface

The findings of Chapter 4 have begun to investigate how key environmental factors influence luxury uptake; however, the polyphosphate in the biomass at any one time is dependent on both accumulation and utilisation. Consequently, further investigation was required. In this chapter batch experiments were used to investigate the influence of key environmental factors on both polyphosphate accumulation and utilisation with respect to time. This further investigates Objective 3 of the thesis which is to determine how key factors influence the luxury uptake mechanism. The results of these findings are then used to propose the conditions required in a new luxury uptake process via microalgae.

This chapter is based on the following publication:

Powell, N., Shilton, A., Pratt, S., & Chisti, Y. (2009). Towards a luxury uptake process via microalgae - Defining the polyphosphate dynamics. *Water Research, 43(17), 4207-4213*

Abstract

Microalgae in waste stabilisation ponds (WSP) have been shown to accumulate polyphosphate. This luxury uptake of phosphorus is influenced by the wastewater phosphate concentration, light intensity and temperature, but the dynamics of how these factors affect luxury uptake with respect to time are not understood. With improved understanding of the dynamics of this mechanism and how it could be manipulated, a phosphorus removal process utilising luxury uptake by microalgae might be developed. In this chapter, luxury uptake was further investigated to determine how the key environmental factors influence both accumulation and utilisation of polyphosphate. The results showed that the initial accumulation and subsequent utilisation of both acid-soluble polyphosphate (ASP) and acid-insoluble polyphosphate (AISP) is a function of the wastewater phosphate concentration. It was found that light intensity influenced both the accumulation and utilisation of ASP. The temperature influenced the accumulation of AISP. AISP is believed to be a form of phosphorus storage and ASP is involved in metabolism however, the results of this work indicate that ASP can also act as a short term form of phosphorus storage. To optimise luxury uptake by microalgae a 'luxury uptake pond' is proposed where the conditions the microalgae are exposed to can be manipulated. This 'luxury uptake pond' would be designed to expose the microalgae to a high phosphate concentration and high light intensity for a short period of time in order to achieve optimal polyphosphate accumulation. Subsequent harvesting would then remove the phosphorus rich microalgae from the system.

5.1 Introduction

Acid-soluble polyphosphate (ASP) is believed to be used for metabolism and production of DNA and proteins. In contrast, acid-insoluble polyphosphate (AISP) appears to be a form of phosphorus storage that can be utilised by the cell when the external phosphorus concentration is limiting for growth (Miyachi & Miyachi, 1961; Miyachi & Tamiya, 1961; Miyachi et al., 1964). To study the luxury uptake mechanism it is necessary to make direct measurements of the internal polyphosphate concentration by using chemical extraction techniques which quantify the ASP and AISP fraction. Unfortunately, as noted by other researchers (Elgavish & Elgavish, 1980; Istvanovics, 1993), the extraction and analysis of polyphosphate from microalgae is a very time

consuming procedure and thus the dynamics of this mechanism are rarely investigated. However, in order to progress towards the development of a new phosphorus removal process the dynamics of the luxury uptake mechanism must be studied.

This chapter investigates the effect of phosphate concentration, light intensity and temperature on the polyphosphate concentration in the microalgae with respect to time. Understanding already exists of how phosphorus moves between the intracellular pools however this work will help to define how these environmental factors influence the dynamics of these transformations. The results of the study then allow possible methods of optimising for phosphorus removal via luxury uptake by microalgae in full scale pond systems to be proposed.

5.2 Materials and methods

5.2.1 Experimental setup

Six litre batch reactors with a length and width of 290 mm and a culture depth of approximately 70 mm were used. Daylight fluorescent tubes (Philips, 36W) were used as a light source and the light intensity was measured at the surface of the reactors using an irradiance sensor (Biospherical Instruments QSL-2101; Biospherical Instruments Inc., San Diego, CA, USA). The reactors were gently mixed using magnetic stirrers (50 mm stirrer bars). All experiments were conducted in temperature controlled rooms. The reactors were regularly weighed and any evaporative losses were corrected for by adding distilled water.

The factors tested were the phosphate concentration in the wastewater, the light intensity and the temperature using the factorial matrix in Chapter 4 (Table 4.1). An additional experiment was conducted in this chapter (Experiment I) which tested a phosphate concentration of 30 mg/L, light intensity of 150 $\mu E/m^2 \cdot s$ and temperature of 25 °C.

5.2.2 Inoculum

A continuous flow reactor (Reactor 2, Chapter 3), initially inoculated with water from a WSP and fed with synthetic wastewater (Davis & Wilcomb, 1967), was used to provide a consistent source of inoculum for the batch experiments. This reactor contained a mixed culture dominated by the microalga *Scenedesmus*. It was allowed to reach steady-state prior to use as an inoculation source for the batch experiments. Full details of this continuous flow inoculum reactor can be found in Chapter 3 (Reactor 2).

5.2.3 Synthetic wastewater

Synthetic wastewater was used to ensure a consistent composition for all experiments. The synthetic wastewater (Davis & Wilcomb, 1967) contained chelating agents to prevent possible removal of phosphorus by precipitation so that the biological uptake mechanisms could be studied. The composition of the synthetic wastewater is given in Chapter 2 (Tables 2.1 and 2.2). At the start of a batch experiment the synthetic wastewater was inoculated with broth from the continuous inoculum reactor. The inoculum volume was 10% of the total liquid volume in the reactor.

5.2.4 Analytical analysis

The phosphate concentration was measured using ion chromatography (Dionex ICS-2000; Dionex Corporation, Sunnyvale, CA, USA) as described in Chapter 2. The microalgal biomass concentration was measured as dry weight using a 0.45 µm filter membrane (Lee & Shen, 2004). All samples were analysed in duplicate.

The polyphosphate fractions in the microalgae were recovered using a series of extraction steps as described by Aitchison and Butt (1973) and Kanai et al. (1965). The extraction steps used trichloroacetic acid, ethanol, ethanol: ether (3:1 by volume) and potassium hydroxide as extraction solvents. The extracts were analysed for total phosphorus to determine the ASP and AISP. Total phosphorus samples were analysed using the sulphuric acid and nitric acid digestion followed by the ascorbic acid colorimetric method in accordance with standard methods (APHA et al., 1995).

5.2.5 Statistical analysis

'Main effects plots' were used to examine the overall effect of the factors tested. These plots show the average effect that each variable has on the polyphosphate content of the microalgae. The main effects plots were generated using MINITAB (Minitab Inc., State College, PA, USA). P-values were then used to determine whether the effect was statistically significant. A p-value less than 0.1 indicates significance at 90% confidence.

5.3. Results and discussion

5.3.1 Effect of phosphate concentration

The findings from Chapter 4 showed that the phosphate concentration did not have a significant effect on luxury uptake. To further investigate the effect of the phosphate concentration on both accumulation and consumption of polyphosphate a larger range of levels were investigated. The phosphate concentrations tested in these experiments were 5, 15 and 30 mg/L although the actual starting phosphate concentrations (as shown in Figure 5.1) were slightly lower than these values because of dilution by the inoculum which contained a lower phosphate concentration.

As shown in Figure 5.1, the initial phosphate concentration in the wastewater had a strong influence on the accumulation of both ASP and AISP in the microalgae. At an initial phosphate concentration of 5 mg/L in the wastewater (Figure 5.1 a), there was accumulation of AISP but no net increase of ASP above the starting value was detected. The AISP is thought to be accumulated by microalgae as a form of phosphorus storage (Miyachi & Miyachi, 1961; Miyachi & Tamiya, 1961; Miyachi et al., 1964) which enables several cell divisions in the absence of any external phosphate (Jansson, 1988; John & Flynn, 2000).

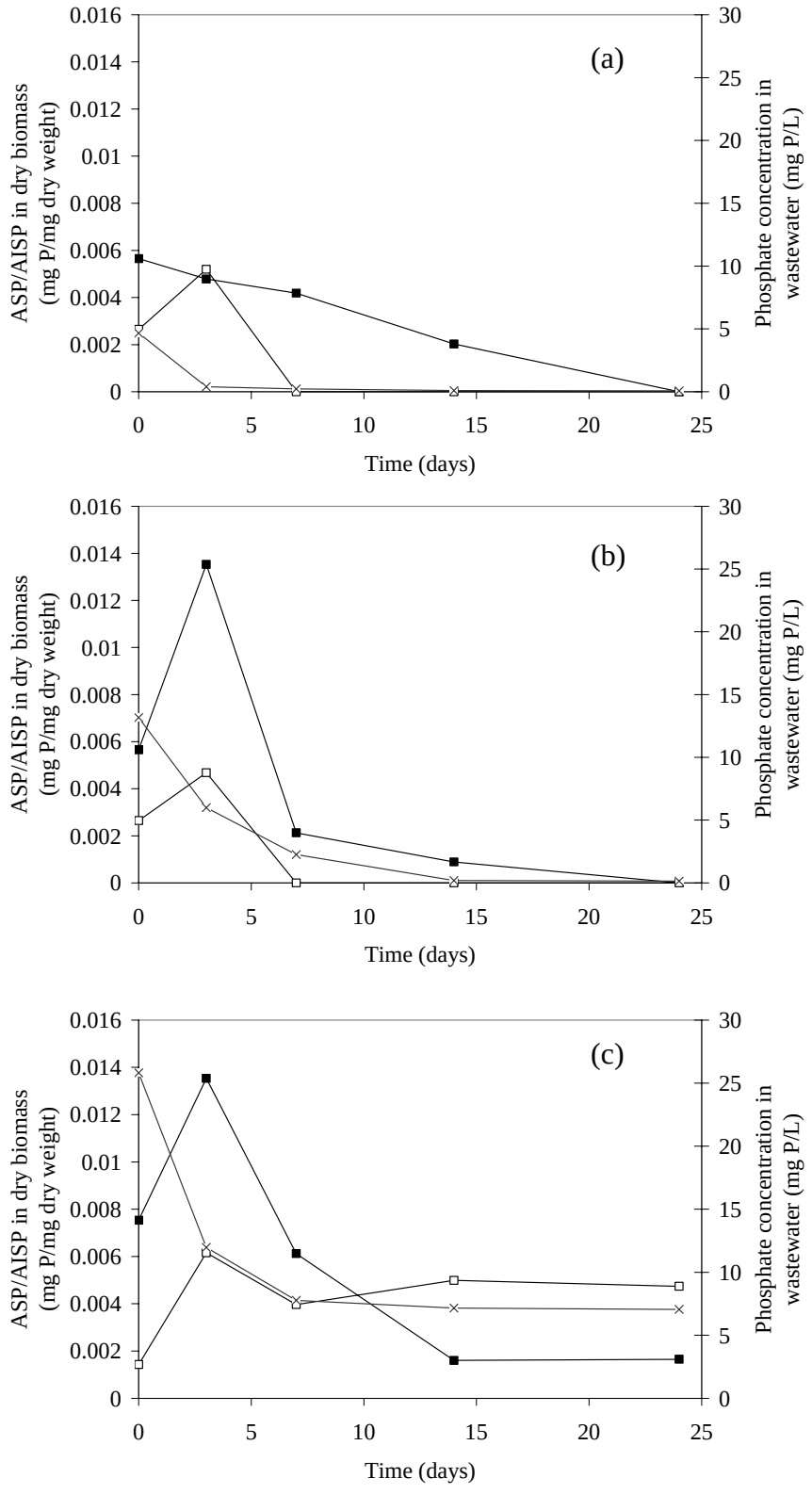

Figure 5.1: Initial phosphate concentration of 5 mg P/L (a), 15 mg P/L (b) and 30 mgP/L (c). ASP per unit dry weight (■), AISP per unit dry weight (□) and phosphate concentration in the wastewater (x).

At higher initial phosphate concentrations of 15 and 30 mg/L (Figure 5.1 b and c) accumulation of both ASP and AISP occurred. These results (Figure 5.1) suggest that while the process of accumulating AISP occurred at all phosphate concentrations tested, net accumulation of ASP occurs at a concentration higher than 5 mg/L, being definitely observed at 15 mg/L.

AISP is known to be a form of polyphosphate which is stored in the cells to act as a phosphorus source when the external phosphate concentration is limiting for growth (Miyachi & Miyachi, 1961; Miyachi & Tamiya, 1961; Miyachi et al., 1964). However, under the conditions tested in these experiments AISP was found to be consumed when phosphate was still present in the synthetic wastewater (Figure 5.1 b). Furthermore, AISP consumption occurred when ASP was present in the cells (Figure 5.1a). This may be due to the transformation of AISP to cellular phosphate. This is discussed in more detail in section 5.3.4.1 of this chapter where an overview of the phosphorus transformations in microalgae is given and the findings of this work are incorporated.

As can be seen in Figure 5.1 c at the highest level of phosphate tested (30 mg/L), polyphosphate was accumulated and only partially utilised by the cells. In fact, Figure 5.1 c shows that after the initial AISP peak there was little change in the AISP concentration. As can be seen in Figure 5.1 c, phosphate was present in the wastewater throughout the experiment which would explain why the AISP was not utilised. Because the rate of phosphate uptake is dependent on the intracellular total phosphorus concentration (Rhee, 1973), the phosphate uptake from the wastewater ceased however, it appears that ASP continued to be utilised as a phosphorus source for growth.

While ASP is known to be involved in metabolism the results of this study indicate that ASP can also act as a form of short term polyphosphate storage as evidenced by the peaks in Figure 5.1 b and c. However, while AISP could be stored over a long period of time as shown in Figure 5.1 c, ASP was rapidly utilised after approximately three days (Figure 5.1).

These batch experiments resulted in the microalgae being exposed to variable phosphate concentrations as phosphate was taken up by the microalgae over time. The data was also examined to determine whether there was a direct relationship between the

phosphate concentration the microalgae were exposed to and the polyphosphate content of the biomass. However, there was no clear relationship which is likely to be due to the dependence on the conditions the microalgae were exposed to previously and whether luxury uptake had previously occurred.

The results indicate that a high phosphate concentration in the wastewater can trigger luxury uptake. To expose microalgae to a maximum phosphate concentration in a full scale application a small separate pond may be required. This will be referred to as a ‘luxury uptake pond’. In this ‘luxury uptake pond’ concentrated microalgal biomass separated from pond effluent, could be directly mixed with the wastewater before the phosphate concentration is diluted in the larger treatment ponds.

5.3.2 Effect of temperature

Temperature affects the rate of all metabolic processes. In addition, temperature may indirectly affect the rate of phosphorus uptake by microalgae by influencing the properties of water, ionic speciation of phosphate, the rate of diffusion across the boundary layer that surrounds the microalgae and so on (Cembella et al., 1983). Figure 5.2 a shows the affect of temperature on the ASP in the microalgae.

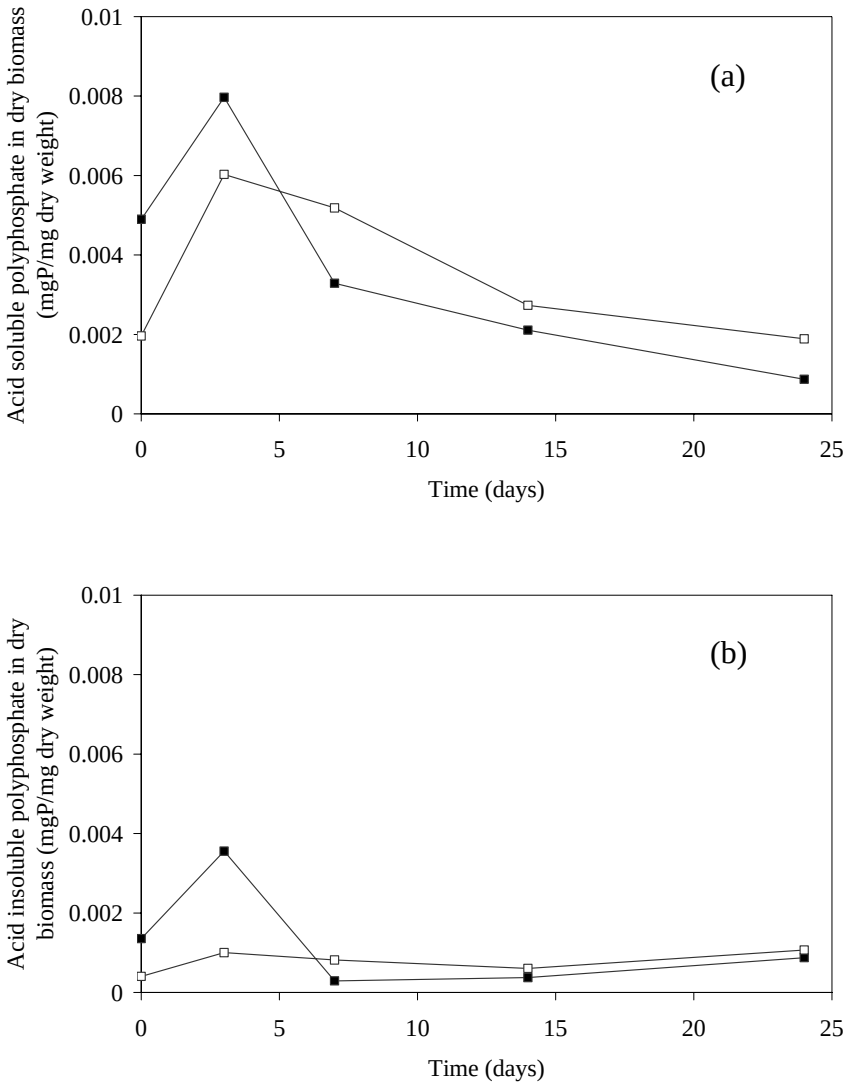

Figure 5.2: 'Main effects plot' of the influence of temperature (25 °C ■, 15 °C □) on the ASP per unit dry weight (a) and AISP per unit dry weight (b).

As might be expected the temperature has some influence on both the accumulation and subsequent consumption of the ASP (Figure 5.2 a). A key difference that can be seen in Figure 5.2 a is that the ASP is consumed more rapidly at the higher temperature (25 °C) than the lower temperature (15 °C). However, the overall effect of temperature on ASP has a p-value of 0.112 which shows that this effect is not significant at 90% confidence.

As shown in Figure 5.2 b temperature also affects the accumulation of AISP. This was confirmed using statistical analysis as the effect of temperature on AISP had a p-value of 0.072 showing that this was significant to 90% confidence. At 25 °C a large initial

peak was observed which was then quickly consumed by the microalgae. However, after seven days the difference in temperature appears to have had little net effect on accumulation of AISP.

The temperature regime of a WSP depends on geographic location and at any given location the temperature varies both daily and seasonally. Although polyphosphate accumulation is influenced by temperature, significant quantities of polyphosphate were accumulated at both of the temperatures tested which suggests that this uptake mechanism will operate under a variety of climatic conditions.

5.3.3 Effect of light intensity

While a number of studies have focused on phosphorus metabolism under constant light or darkness (for example Kylin, 1966), few studies apart from those presented in this thesis have assessed the influence of different light intensities. The effect of light intensity on the ASP and AISP content of the microalgae is shown in Figure 5.3.

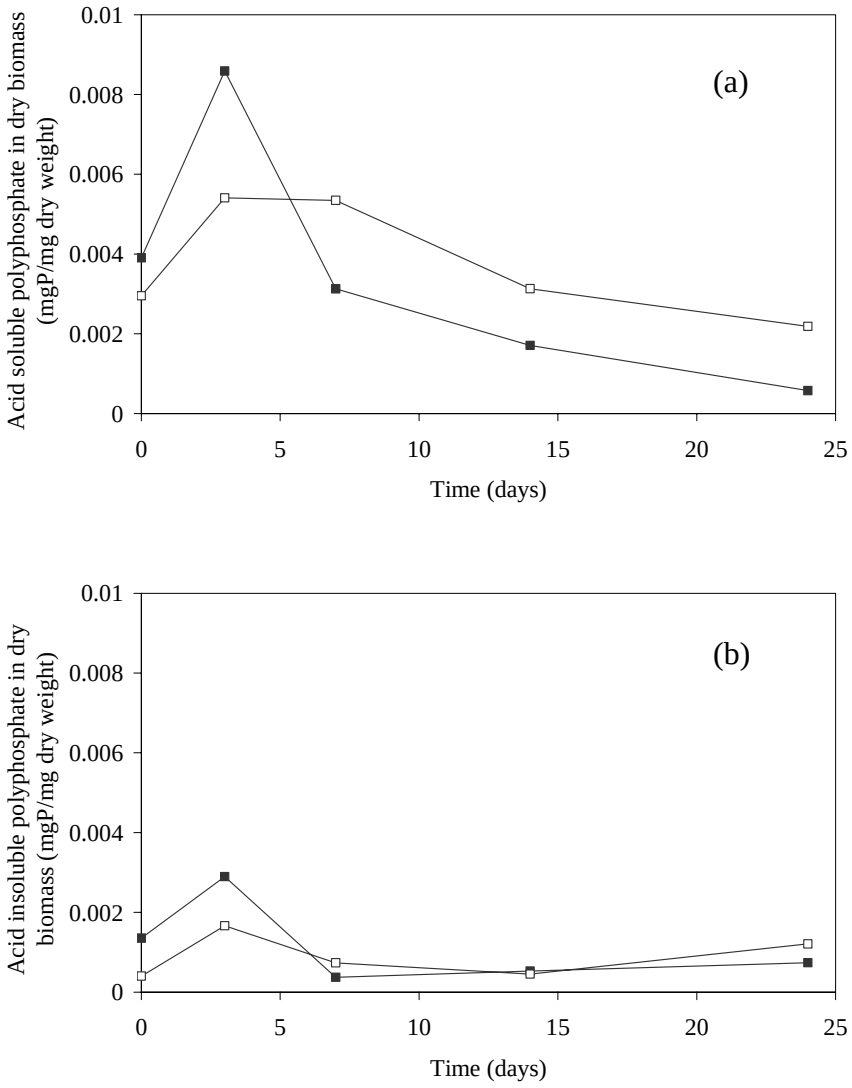

Figure 5.3: 'Main effects plot' of the influence of light intensity (150 µE/m^2·s ■, 60 µE/m^2·s □) on the ASP per unit dry weight (a) and AISP per unit dry weight (b).

Light intensity was shown to not have any major effect (p-value 0.466) on the AISP in the microalgae (Figure 5.3 b). Figure 5.3 a shows the effect of light intensity on the ASP in the microalgae.

Light intensity had a significant effect on ASP with a p-value of 0.077 indicating 90% confidence. Higher light intensity (150 µE/m^2·s) resulted in a higher amount of ASP initially accumulated in the microalgae; however, at high light intensity it appears that the microalgae consumed the polyphosphate rapidly (Figure 5.3 a). While Figure 5.3 a shows that ASP accumulation was slower under low light (60 µE/m^2·s) subsequent consumption was also delayed, possibly due to a slower microalgal growth rate.

Because of this delay in consumption, after approximately five days the amount of ASP stored in the microalgae was actually higher at the low light intensity (60 μE/m^2·s) than at the high light intensity (150 μE/m^2·s) (Figure 5.3 a).

It is important to note that as microalgae grow mutual shading occurs which would have resulted in a decreasing light intensity throughout the depth of the reactor according to the Beer-Lambert Law. This means that the light intensity that the microalgal cells were exposed to would have changed with time. Due to the well mixed reactors the microalgae would have been exposed to a dark/light cycle as a result of moving between the well illuminated surface and the darker zone near the base of the reactor. The data in this chapter has been analysed in terms of the light intensity at the surface of the reactor. Further research would be needed to determine whether the light cycle that the microalgae are exposed to influences luxury uptake.

It has been shown that light intensity has a significant impact on accumulation of ASP. This factor could therefore potentially be manipulated in a 'luxury uptake pond' to optimise phosphate uptake. In pond systems the light intensity reduces rapidly with increasing depth due to absorption as a result of high levels of suspended solids and the presence of humic matter. Consequently, the exposure of microalgae to light decreases with depth. Increasing vertical mixing in a 'luxury uptake pond' would increase the amount of light that the microalgal cells are exposed to and therefore might potentially provide a technique for optimising the rate of polyphosphate accumulation.

The hydraulic retention time of the 'luxury uptake pond' also needs to be considered if polyphosphate accumulation is to be optimised. As shown in Figure 5.3 a, sufficient time is needed for luxury uptake to occur, however, if the microalgae are exposed to light over a long period of time the accumulated polyphosphate is consumed for growth.

5.3.4 Summary of findings

The two main outcomes of the research presented in this chapter are firstly an enhanced understanding of how the dynamics of the polyphosphate pools within microalgae are influenced by key environmental factors. Secondly, with understanding of these factors,

a number of considerations that could lead to the development of a process utilising luxury uptake by microalgae can be proposed.

5.3.4.1 Defining the dynamics of the polyphosphate pools

The range of mechanisms which are occurring simultaneously, some of which are interdependent, make understanding the dynamics of polyphosphate accumulation and utilisation in microalgae very complex. In Figure 5.4 the findings reported in this paper have been summarised to illustrate the transformations that occur between the different phosphorus pools and the factors that affect these transformations. While the biological transformations in Figure 5.4 are based on previous literature it is the work presented in this thesis that allows the key factors which affect the net transfer of phosphorus in and out of the polyphosphate pools to be identified.

The intracellular phosphorus can be used by the microalgae for a number of processes. The two main pathways are polyphosphate production and the production of substances such as phospholipids or RNA which are required for metabolism. This means that the net amount of phosphorus available for polyphosphate production is dependent on the rate of phosphate uptake across the cell wall and the subsequent use of phosphorus for growth.

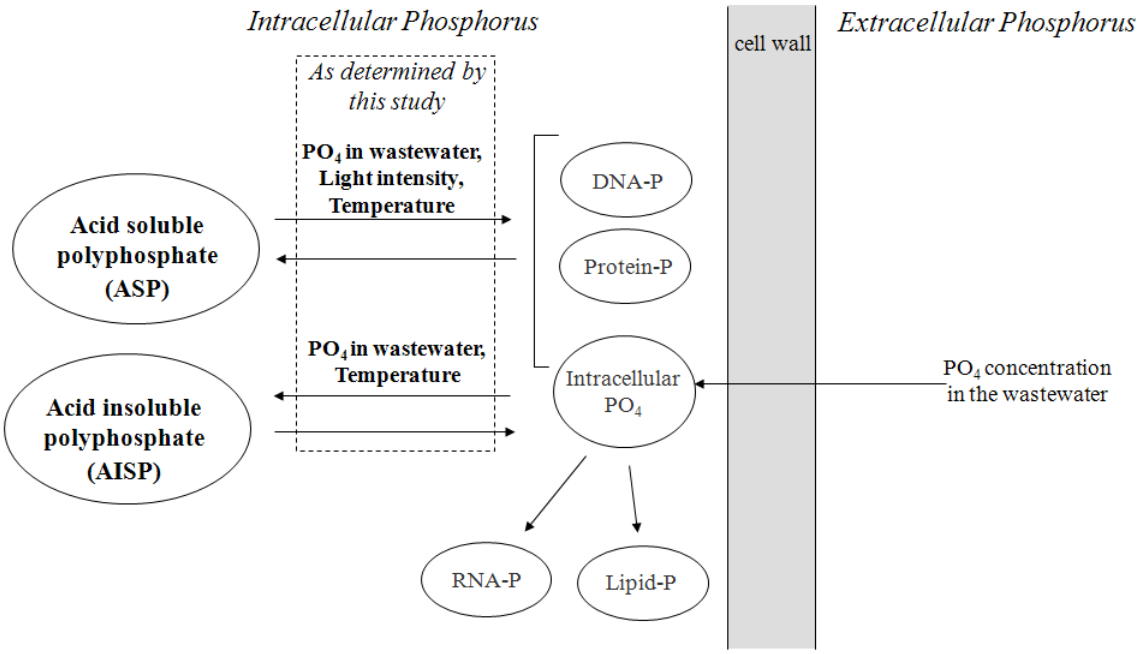

Figure 5.4: Summary diagram of how factors affect the polyphosphate pools in WSP microalgae. Transformations between pools are adapted from Miyachi et al. (1964).

The AISP pool is used by the microalgae as a phosphorus store and consumption was thought to occur when the external phosphate concentration reaches a growth limiting level (Miyachi et al., 1964). However, the data presented in this chapter suggests that under the conditions tested in these experiments once AISP consumption was triggered the AISP decreased rapidly irrespective of the phosphate concentration in the synthetic wastewater or presence of ASP. This rapid consumption of AISP may be due to the AISP being transformed into intracellular phosphate (Figure 5.4) which could then in turn have been rapidly utilised for the formation of several different cellular constituents. The consumption of AISP in the presence of external phosphate or ASP does not appear to have been reported in previous research which may have been because this work has only focussed on natural systems such as lakes where the phosphate concentrations are much lower than WSP systems. The findings of this thesis also show that the AISP in the microalgae is influenced by the temperature and the phosphate concentration in the wastewater (Figure 5.4).

As illustrated in Figure 5.4 the ASP pool is used as a source of phosphorus for DNA and protein production but this polyphosphate can also become available for other processes when the phosphate in the wastewater reaches a certain concentration where growth is limited (Miyachi et al., 1964). The findings of this thesis have shown that the ASP in the microalgae is influenced by the phosphate concentration in the wastewater, light intensity and temperature.

While AISP is known to be a form of phosphorus storage, the findings of this chapter suggest that ASP can also be accumulated within the microalgae as a form of short term phosphorus storage when exposed to high phosphate concentrations in the surrounding wastewater. However, as ASP is subsequently used for growth this form of polyphosphate is then rapidly consumed. The ability for ASP to provide short term phosphorus storage may have not been detected in previous studies as that research focused on microalgae natural ecosystems where phosphate concentrations are significantly lower than those found in WSP.

5.3.4.2 Towards a phosphorus removal process

The relatively low microalgal biomass concentration typically found in WSP means that biological phosphorus uptake in WSP is generally low. However, if luxury uptake is triggered phosphorus uptake from the wastewater could be more than tripled as discussed in Chapter 4. Additionally if the microalgal biomass concentration was increased, very high levels of biological phosphorus uptake might then be achieved. Harvesting of the phosphorus rich microalgal biomass would then enable enhanced net phosphorus removal.

While the previous chapters had determined the key factors that influence luxury uptake (Chapter 4) understanding of the dynamics of the luxury uptake mechanism studied in this chapter now enables the environmental conditions required to trigger luxury uptake to be proposed. This is an important step to allow a new phosphorus removal process via luxury uptake by microalgae to be developed. A 'luxury uptake pond' could be developed where the conditions the microalgae are exposed to are manipulated to optimise luxury uptake by microalgae. In developing this process the following might be considered:

- Concentrated microalgae collected by liquid/solids separation of the effluent from the main pond system could be mixed with wastewater before it is diluted in the larger ponds. This wastewater has the highest phosphate concentration in the system which, as experimental results show, maximises polyphosphate accumulation (Figure 5.1).

- The experimental results (Figure 5.3 a) indicate that higher light intensity results in rapid polyphosphate accumulation. Therefore, the 'luxury uptake pond' would ideally be vertically mixed to ensure the bulk microalgal biomass is exposed to maximum light intensity.

- The phosphorus rich microalgae leaving the 'luxury uptake pond' would then be harvested with the liquid continuing onto further treatment in the main pond system.

- As a minimum, the retention time of the ‘luxury uptake pond’ should be sufficient for optimal polyphosphate accumulation. But because higher light intensity also promotes microalgal growth, the maximum retention time should limit subsequent consumption of the polyphosphate thereby providing for a minimised yield of biomass with a maximised phosphorus content.

It should be noted that the complexity of transformations between the phosphorus pools combined with the range of influencing factors does make developing a luxury uptake process challenging. Before the concept of a ‘luxury uptake pond’ can be fully proven further research is required using an integrated, bench-top and/or pilot scale reactor.

5.4 Conclusions

The accumulation and utilisation of both ASP and AISP were found to be influenced by the phosphate concentration in the wastewater. Light intensity influenced both the accumulation and utilisation of ASP. At higher light intensity the initial accumulation of ASP was higher; however, the ASP was then rapidly consumed. This resulted in higher ASP in the microalgae at the lower light intensity after approximately five days. The temperature influenced the accumulation of AISP. While previous research had shown that AISP is a form of phosphorus storage, the findings of this paper indicate that ASP can act as a form of short term phosphorus storage.

The key to developing a new algal luxury uptake process would be via manipulation of these factors. Because polyphosphate accumulation was found to increase with an increase in the phosphate concentration, luxury uptake might be optimised by exposing the microalgae to the higher phosphate concentration present at the start of the treatment process in a separate ‘luxury uptake pond’. The retention time of this pond would aim to provide sufficient time for optimal polyphosphate accumulation while limiting subsequent microalgal growth thereby allowing a minimised yield of biomass with a maximised phosphorus concentration. The final step would be to harvest the phosphorus rich microalgae.

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Chapter 6

Phosphorus release from WSP sludge

Chapter preface

Net phosphorus removal in WSP is dependent on the amount of phosphorus that is retained in the sludge. The findings of the previous chapters indicate that luxury uptake can occur in microalgae under WSP conditions. Consequently, the microalgae which settle into WSP sludge could potentially contain significant quantities of polyphosphate. However, it has not previously been investigated whether polyphosphate would be released from microalgal biomass in WSP sludge. This chapter investigates Objective 4 which was to determine the rate of phosphate release from WSP sludge and to investigate whether polyphosphate is released.

This chapter is based on the following publication:

Powell, N., Shilton, A., Pratt, S., & Chisti, Y. Phosphate release from waste stabilisation pond sludge - Significance and fate of polyphosphate. *To be submitted.*

Abstract

Net phosphorus removal within waste stabilisation ponds (WSP) is governed by the rate of phosphorus incorporation into the sludge layer and the rate of phosphorus release from this sludge back to the overlying wastewater. Luxury uptake of phosphorus by microalgae has been shown to occur under WSP conditions in the laboratory; however, the significance of this mechanism and the fate of polyphosphate contained in the settled biomass have not previously been investigated. In this work the analysis of sludge samples from three WSP showed that up to 71% of the total phosphorus in the sludge was in the form of polyphosphate. This indicates that polyphosphate accumulation could potentially be an important mechanism for phosphorus removal in WSP and challenges the dogma that chemical precipitation is the predominant phosphorus removal mechanism in these systems. The release of phosphate from WSP sludge samples was monitored in the laboratory. The samples from two different pond systems had initial release rates in the order of 4.3 µgP/gTSS.d. However, the third sample which was collected during an algal bloom had a release rate of 12.4 µgP/gTSS.d. Phosphate release from fresh microalgal sludge grown under laboratory conditions was also studied and was shown to have an initial release rate of 160 µgP/gTSS.d. Analysis of polyphosphate during the experiments on laboratory grown microalgal sludge showed that polyphosphate was indeed degraded resulting in phosphate being released. Interestingly, after the initial release phase phosphorus was uptaken by the biomass and some polyphosphate was reformed. It is likely that this is due to bacterial growth in the sludge.

6.1 Introduction

The amount of phosphorus removed within WSP is dependent on two processes – the rate of incorporation of phosphorus into the sludge and the rate of phosphorus release from the sludge. Phosphorus can settle into the sludge layer due to the primary settling of solids, precipitation and adsorption reactions and settling of microalgae, bacteria and other organisms. A large amount of phosphorus can be accumulated in the sludge; however, some of this phosphorus is later released. The exchange of phosphorus between the sludge and the overlying wastewater is a complex process which involves physical, chemical and biological processes. The rates of these processes are influenced

by environmental conditions such as pH, temperature, redox potential and biological activity (Ortuno et al., 2000).

Studying the different forms of phosphorus in WSP sludge is vital to understand the phosphorus removal mechanisms that occur in WSP. Only a limited number of studies exist on phosphorus in WSP sludge (Carre & Baron, 1987; Gomez et al., 2000; Ortuno et al., 2000; Peng et al., 2007) and the majority of these studies have focused on chemical precipitates present in the sludge. This is because chemical precipitation is commonly believed to be the predominant phosphorus removal mechanism (Shelef et al., 1982; Picot et al., 1991; Moutin et al., 1992; Nurdogan & Oswald, 1995). Other studies disagree with this existing dogma that chemical precipitation is the most significant mechanism and have found that removal by biological assimilation appears to dominate (Cromar et al., 1992; Mesple et al., 1995) at least in some cases.

Microalgae can accumulate phosphorus within their cells as polyphosphate. This mechanism known as luxury uptake is not usually considered in studies of phosphorus removal in WSP; however, it has been shown to occur in WSP microalgae as discussed in Chapters 3–5. Consequently, the microalgae which settle into WSP sludge could potentially contain significant quantities of polyphosphate. However, it is not currently known whether this polyphosphate is released from WSP sludge and the rate at which phosphorus release occurs.

The purpose of this chapter was to determine the significance and fate of polyphosphate in WSP sludge and to determine the rate of phosphorus release from WSP sludge. Both WSP sludge and laboratory grown microalgal sludge was analysed.

6.2 Methods

6.2.1 Field WSP sludge samples

Sludge samples were collected from WSP located in Ashhurst, Aokautere and Foxton Beach. All these pond systems are in the lower part of the North Island, New Zealand and treat domestic wastewater. The WSP sampled all consist of two ponds in series, but

sludge samples were only taken from the second pond. This is because the first pond contains settled solids from the raw wastewater which are not the focus of this chapter. Sludge samples were analysed and the phosphate release experiments were started within three hours of sampling. The sludge was collected as 'grab samples' which comprised of approximately the top 10 cm of the sludge layer. This is because sludge lower than this is likely to have released a significant portion of the phosphorus it originally contained.

6.2.2 Laboratory microalgal sludge samples

A continuous culture inoculum reactor dominated by the microalga *Scenedesmus* was used for the microalgal sludge sample (Reactor 2, Chapter 3). The effluent was collected and allowed to settle to form a microalgal sludge. Further details of this reactor have been previously reported in Chapter 3.

6.2.3 Phosphate release experiments

Measuring cylinders (500 ml) were used to conduct the phosphate release experiments. The cylinders were filled with 100mL of sludge and 400 ml of liquid. The liquid added was either filtered pond water for the field sludge experiments, or filtered effluent from the continuous reactor for the laboratory microalgal sludge experiments. This was done to ensure that the chemical composition of the liquid surrounding the sludge did not change. The liquid was filtered using a 0.45 µm membrane filter. The measuring cylinders were completely covered with tin foil to prevent exposure to light and subsequent microalgal growth. All experiments were conducted in a controlled temperature room at 25 °C. The experimental setup is shown in Figure 6.1.

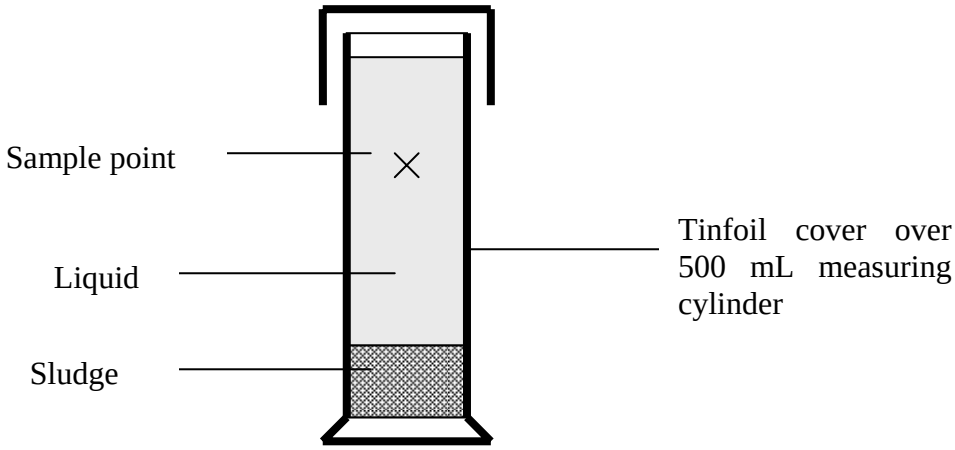

Figure 6.1: Phosphate release experimental setup

Phosphate release experiments were conducted for each of the field WSP sludge samples. To study the types of phosphorus released by the laboratory microalgal sludge in more detail, two phosphate release experiments were conducted using laboratory grown microalgal sludge. The first experiment monitored the initial release phase and was run for 50 days. The second experiment studied the long term phosphate release and was monitored for 250 days.

6.2.4 Analytical methods

The initial phosphorus content of the sludge was measured at the start of the phosphate release experiments to determine the types of phosphorus present and give insights into the phosphorus removal mechanisms in WSP. The polyphosphate was analysed using the extraction methods described by Aitchison and Butt (1973) and Kanai et al. (1965). This procedure used extractions with trichloroacetic acid, ethanol, ethanol: ether (3:1 by volume) and potassium hydroxide. Total phosphorus analysis was conducted according to standard methods (APHA et al., 1995) using the nitric and sulphuric acid digestion and analysis using the ascorbic acid method. These measurements were repeated at the end of the experiment for the laboratory microalgal sludge. The total suspended solids concentration (TSS) of the sludge samples were measured according to standard methods (APHA et al., 1995) using Whatman GF/C filter papers. All samples were analysed in duplicate.

Samples were taken from the liquid regularly to monitor the rate of phosphate release. The samples were taken at a depth of 15 cm from the rim of the measuring cylinder. The liquid samples were analysed for phosphate in duplicate using ion chromatography (Dionex ICS-2000; Dionex Corporation, Sunnyvale, CA, USA) as described previously in Chapter 2.

6.3 Results and discussion

6.3.1 Polyphosphate in field WSP sludge

The phosphorus removal mechanisms that naturally occur in WSP include both biological uptake and chemical precipitation. Ultimately these mechanisms result in biomass or precipitates settling into the sludge layer. Therefore, analysing WSP sludge samples for the different phosphorus fractions gives indications of the phosphorus removal mechanisms that are occurring. WSP sludge samples were collected at three WSP and analysed for the different phosphorus fractions present. The phosphorus fractions found in the samples are shown in Figure 6.2.

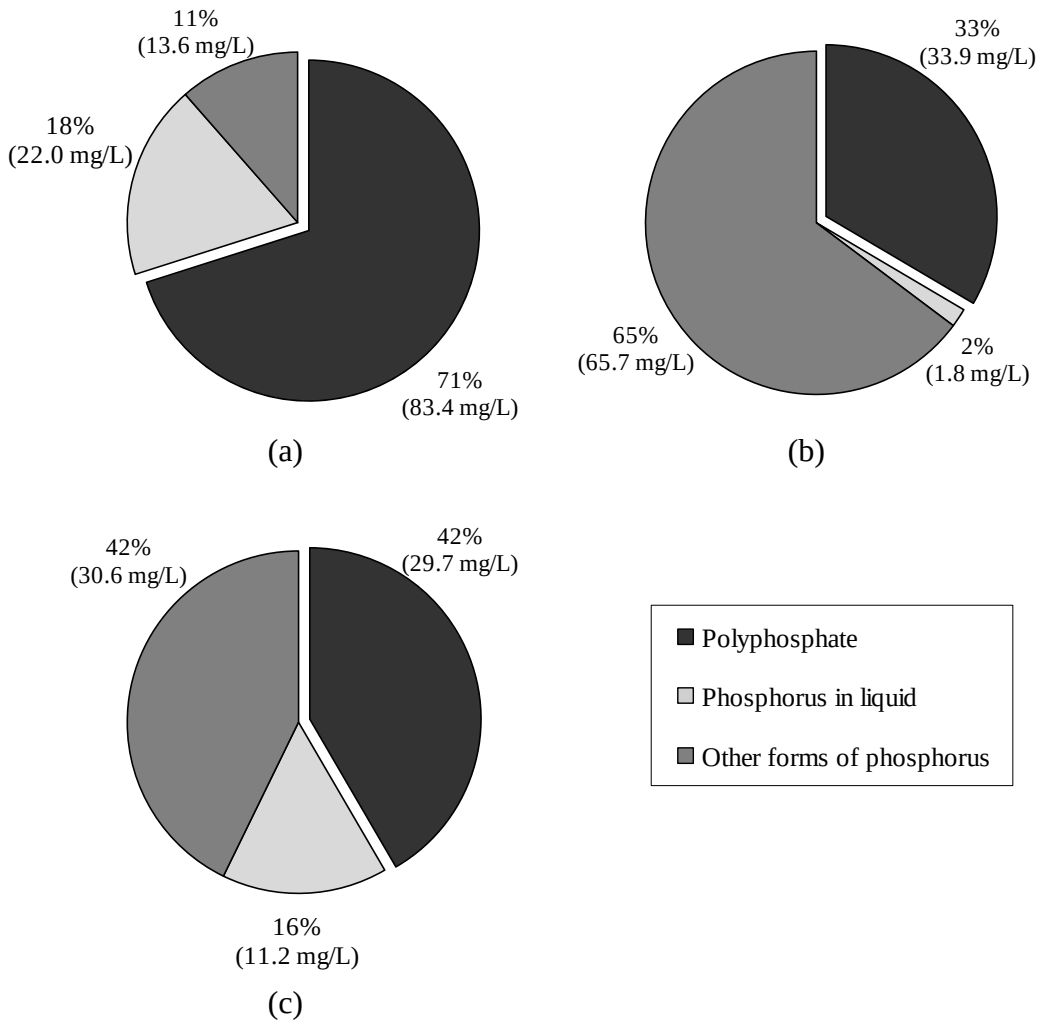

Figure 6.2: Phosphorus fractions as a percentage of the total phosphorus in the WSP sludge samples from Ashhurst (a), Aokautere (b) and Foxton Beach (c). The values shown in brackets give the concentration for each of the fractions.

As can be seen in Figure 6.2 significant amounts of polyphosphate are present in each of the field WSP sludge samples. Polyphosphate accounted for between 33 and 71% of the total phosphorus present (Figure 6.2). This finding certainly questions the validity of the existing dogma that chemical precipitation is the dominant phosphorus removal mechanism in WSP.

Polyphosphate has been previously detected in WSP sludge. Gomez et al. (2000) studied the different types of phosphorus present in pond sludge and while polyphosphate was detected, it was in very small amounts. However, the sludge samples tested by Gomez et al. (2000) had a very low organic phosphorus fraction accounting for only 6–8% of the total phosphorus in the sludge. This very low organic phosphorus

content of the sludge is difficult to explain as the organic phosphorus content of WSP sludge has been reported to account for 27% of the total phosphorus in other studies (Ortuno et al., 2000).

Phosphorus present in the sludge excluding polyphosphate is referred to as other forms of phosphorus. This fraction would consist of both organic and inorganic forms of phosphorus. This means that the quantity of other forms of phosphorus is likely to be variable (as seen in Figure 6.2) due to its dependence on a number of processes. This fraction of phosphorus was not further analysed as this chapter is focused on the significance and fate of the polyphosphate fraction in the sludge.

6.3.2 Phosphate release from WSP sludge

Net phosphorus removal in WSP is influenced by the rate of phosphorus release from the sludge. The phosphate release for each of the three WSP sludge samples was measured in the laboratory. The results are shown in Figure 6.3. This phosphate release data is reported per unit of total suspended solids to correct for differences in the solids concentration between the different sludge samples.

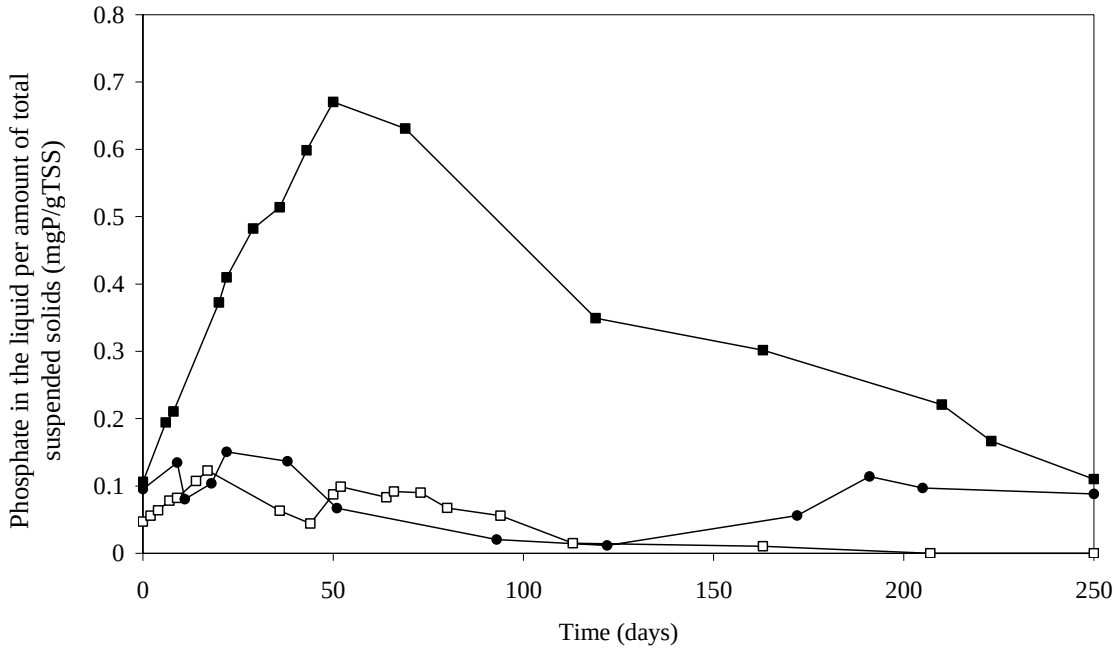

Figure 6.3: Phosphate release from the field WSP sludge samples from Ashhurst (■), Aokautere (□) and Foxton Beach (●).

The sludge sample taken at Ashhurst showed an initial rapid release of phosphate at a rate of 12.4 µgP/gTSS.d (Figure 6.3). As clearly shown in Figure 6.3, the Ashhurst WSP sludge sample released much more phosphate than the samples from the other WSP. A likely explanation for this is that the Ashhurst sample had been taken soon after an algal bloom. At the time the Ashhurst sample was taken the suspended solids concentration at the surface of the pond was three times the average suspended solids measured over a 12 month period. Therefore, the Ashhurst sample probably contained a large amount of fresh microalgal biomass compared to the other WSP sludge samples which would have contained older sludge that would have already started releasing phosphate.

The initial release rate for the Aokautere sludge was approximately 4.3 µgP/gTSS.d for the first 17 days. After this initial release period the phosphate concentration in the liquid decreased indicating that phosphate was being reincorporated into the sludge. Eventually no phosphate was detectable in the liquid. The sludge from Foxton Beach WSP showed a similar trend to Aokautere, with both periods of release and uptake

(Figure 6.3). Possible reasons for the uptake of phosphate are discussed later in this chapter.

Ortuno et al. (2000) conducted phosphate release studies in the laboratory using sludge from a deep WSP and found that phosphate was released at a rate of approximately 125 µgP/gTSS.d. This is ten times higher than the rate of release from Ashhurst (Figure 6.3). The work by Ortuno et al. (2000) also showed that phosphate release occurred for approximately five days however, the results presented in this chapter shows phosphate was released over several months. The likely reason for these differences between the two studies is that the sludge samples used by Ortuno et al. (2000) were freeze dried and homogenised which may have caused cell lysis and therefore accelerated the rate of phosphate release. This illustrates the importance of sludge handling and any pre-treatment when conducting phosphorus release studies.

6.3.3 Phosphate release from microalgal sludge

To assess the rate of phosphate release form fresh microalgal sludge, phosphate release experiments were conducted using laboratory grown microalgal sludge. Figure 6.4 shows the phosphate release results for the initial release experiment along with the long term study. The phosphate release is reported per unit of total suspended solids so the results can be directly compared to the field sludge samples (Figure 6.3).

The initial rapid phosphate release from the laboratory microalgal sludge occurred at a rate of 160 µgP/gTSS.day. Phosphate release occurred for approximately four months. The initial rapid phosphate release and general trend seen in Figure 6.4 is similar to the phosphate release from the Ashhurst sludge sample (Figure 6.3). However, the rate of release at Ashhurst is lower as the field sludge sample would also contain older sludge that had already begun releasing phosphorus before the sample was taken.

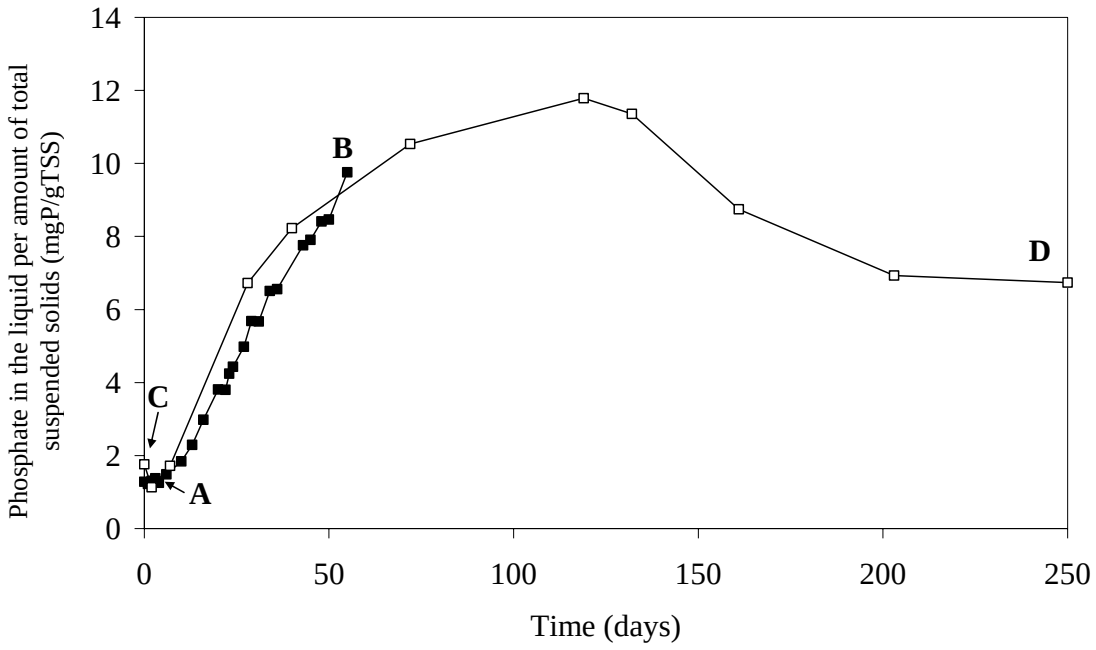

Figure 6.4: Phosphate release from the microalgal sludge for the short term experiment (A to B, shown by ■ symbol) and long term study (C to D, shown by □symbol). The letters A–D correspond to bars shown in Figure 6.5.

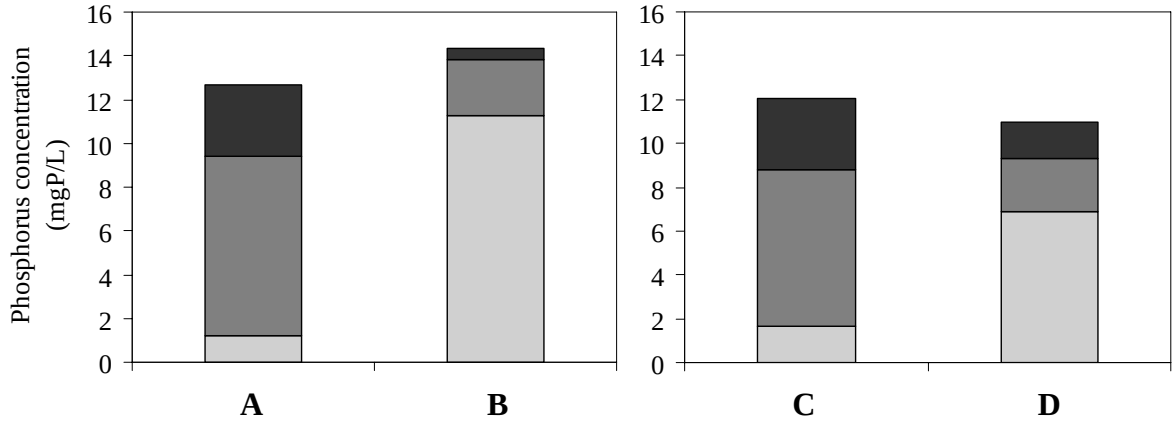

Figure 6.5: Phosphorus fractions in the microalgal sludge for the initial (A and B) and long term (C and D) experiments. The letters A–D correspond to points shown on Figure 6.4. Light grey shows the phosphorus in the liquid, medium grey shows the other forms of phosphorus in the total suspended solids and black indicates the polyphosphate in the total suspended solids.

6.3.4 Type of phosphorus released from microalgal sludge

To understand the phosphorus transformations in WSP sludge and to determine the fate of polyphosphate in the sludge the type of phosphorus released must also be studied. For the two microalgal sludge phosphate release experiments (shown in Figure 6.4) the phosphorus fractions present in the sludge at the start and end of the experiments are shown in Figure 6.5. The initial phosphorus fractions (Figure 6.5, A and C) showed that the samples contained similar amounts of phosphorus including polyphosphate. The sum of the fractions at the start and end of the experiments differ by 10-12%. This difference is likely to be due to the combined error in the total phosphorus analysis in each of the three fractions (liquid, polyphosphate and other forms of phosphorus in the sludge). Another contributing factor would be the non-homogeneous consistency of the sludge.

At the end of the initial phosphate release experiment (Figure 6.5, B), the total amount of phosphorus remaining in the sludge (shown as the sum of polyphosphate and other forms of phosphorus in the sludge) had decreased as expected. Comparing Figure 6.5 A and B shows that the phosphate release (shown in Figure 6.4) was due to the release from both the polyphosphate and other phosphorus fractions in the sludge. This confirms that polyphosphate is indeed degraded resulting in phosphate being released from the sludge.

The phosphorus fractions present at the end of the long term experiment is shown in Figure 6.5, D. The phosphate release data for this experiment (Figure 6.4) shows that the initial rapid release phase resulted in 95% of the phosphorus that was originally in the microalgal biomass being released, which would include a significant amount of polyphosphate. As shown in Figures 6.3 and 6.4, phosphate was also removed from the liquid phase which was an unexpected finding. It was expected that decay would slowly continue, however it appears that uptake has occurred resulting in a net reduction in phosphate in the liquid. The phosphorus fractionation (Figure 6.5, D) indicates that polyphosphate may have been reformed. This may have contributed to the high levels of polyphosphate measured in the field sludge samples (Figure 6.2). The reformation of polyphosphate was unexpected as the experiment was conducted in the dark and microalgal uptake could not have occurred unless the microalgae were capable of

heterotrophic growth. While further investigation is required, it is possible the polyphosphate reformation in the sludge could be due to growth and luxury uptake by bacteria as studies of lake sediments have shown that bacteria present in sediment samples can contain polyphosphate granules (Hupfer et al., 1995). While this may explain the phosphorus uptake in these experiments, it is important to note that these bacteria would not explain the phosphorus uptake reported in the pervious chapters due to the highly aerobic conditions present in the microalgal reactors which do not select for this type of bacteria (Smolders et al., 1994).

6.3.5 Practical implications

This research shows when microalgae settle into the sludge layer phosphorus is released including polyphosphate. This highlights the need to remove microalgae from the WSP if effective phosphorus removal is to be achieved.

These results also have implications for other microalgal based wastewater treatment technologies such as high rate algal ponds (HRAP). HRAP systems typically have an algal settling pond to remove the microalgae from the wastewater. The frequency of emptying these ponds varies but is typically every 4-6 months (Craggs, 2005). The findings from this work indicate that the algal settling pond should be emptied more regularly to prevent the release of phosphate from the microalgal sludge. In light of the findings of this research this simple operational modification will very likely improve phosphorus removal efficiency in these systems.

6.4 Conclusions

Analysis of the phosphorus fractions of the WSP sludge showed that 33–71% of the total phosphorus was in the form of polyphosphate. This indicates that polyphosphate accumulation is an important phosphorus removal mechanism in WSP.

Phosphate release experiments conducted using field WSP sludge showed phosphate release rates of 12.4 µgP/gTSS.d occurred at a WSP after an algal bloom whereas the other WSP had rates of approximately 4.3 µgP/gTSS.d. The rate of phosphate release

from fresh microalgal sludge was 160 µgP/gTSS.d which defines the high potential rate of release from fresh microalgal sludge.

Analysis of the phosphorus fractions in the laboratory microalgal sludge showed that polyphosphate was indeed degraded which resulted in phosphate being released. These findings show that there is a limited storage period for the microalgal sludge.

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Chapter 7

Luxury uptake in full-scale WSP

Chapter preface

This chapter is focused on the last objective of the thesis which is to investigate the significance of biological phosphorus uptake in WSP and to determine whether luxury uptake occurs in full-scale WSP. The majority of the data presented in the previous chapters is based on studies conducted in the laboratory. These laboratory studies are vital for studying the biological phosphorus uptake mechanisms because the environmental conditions need to be carefully controlled. However, field studies are also required to confirm that these mechanisms do indeed occur in full-scale WSP.

This chapter is based on the following publication:

Powell, N., Shilton, A., Pratt, S., & Chisti, Y. (2009). Luxury uptake of phosphorus by microalgae in full-scale waste stabilisation ponds. *Paper presented at the Water New Zealand Conference, Water 2020: From fragmentation to efficiency, Rotorua.*

Abstract

Biological phosphorus uptake was studied in two full-scale waste stabilisation ponds (WSP). Luxury uptake by microalgae was confirmed to occur and in one pond the biomass contained almost four times the phosphorus required by microalgae for normal metabolism. However, the phosphorus content within the biomass was variable. This finding means that assumptions made in prior publications on modelling of phosphorus removal in WSP are questionable. While fluctuations in microalgal growth causes variation in many water quality parameters, this further variation in luxury uptake explains the high degree of variability in phosphorus removal commonly reported in the literature. To achieve effective biological phosphorus removal high levels of both luxury uptake and microalgal concentration are needed. The findings of this work show that while high levels of these parameters did occur at times in the WSP monitored, they did not occur simultaneously. This is explained because accumulated phosphorus is subsequently consumed during rapid growth of biomass resulting in a high biomass concentration with a low phosphorus content. Previous laboratory research has allowed a number of key considerations to be proposed to optimise both luxury uptake and biomass concentration. Now that is has been shown that high levels of biomass concentration and luxury uptake can occur in the field it may be possible to redesign WSP to optimise these parameters. If this was possible biological phosphorus removal from the effluent in the order of 75–100% could potentially be achieved.

7.1 Introduction

Phosphorus removal in WSP is highly variable. For example, a study conducted in Brazil found that the effluent from a four pond system contained a minimum total phosphorus concentration of 6.6 mg/L and a maximum of 12.0 mg/L (Bento et al., 2002). A similar amount of variation was also reported by Davies-Colley et al. (1995) when ten WSP systems in New Zealand were studied. The 5^{th} and 95^{th} percentile values for total phosphorus concentration were 3.5 mg/L and 9.7 mg/L, respectively. While it is encouraging that high levels of phosphorus removal have, at times, been achieved it is not clear why this fluctuation in removal occurs or how a high level of phosphorus removal can be sustained. To determine why this variation occurs and how to improve

phosphorus removal in WSP, the mechanisms responsible for phosphorus removal need to be understood.

Phosphorus removal in WSP is known to occur due to chemical precipitation of phosphorus and biological assimilation. While the naturally occurring mechanisms of chemical precipitation have been investigated (for example Moutin et al., 1992; Nurdogan & Oswald, 1995) few researchers have focused on the biological phosphorus removal mechanisms in WSP. Microalgae and other organisms in WSP require phosphorus as a nutrient for growth. Microalgae have been reported to make up the largest organic phosphorus pool in the water column of pond systems (Pearson, 2005). Phosphorus uptake by algae is largely thought to be due to its uptake for normal growth. However, uptake by luxury uptake may also be occurring. Luxury uptake, which involves storage of phosphorus in the form of polyphosphate, has been shown to occur in WSP microalgae studied under laboratory conditions in the previous chapters. However, the question remains whether such a phenomenon is present in full-scale field WSP and if so to what extent.

This chapter reports on the occurrence of luxury uptake of phosphorus in two full-scale WSP. The potential biological phosphorus removal in the two WSP if luxury uptake was optimised is then investigated.

7.2 Methods

Sampling was conducted at the Ashhurst and Aokautere WSP which are in the Manawatu region of New Zealand. Both of these systems treat domestic wastewater. Details of these WSP are given in Table 7.1. In each case both the facultative and maturation ponds were sampled from a consistent location near the effluent pipe of each pond. ‘Grab samples’ were taken from the top 10 cm of the pond to ensure that the sample was taken within the algal band as this study focused on biological phosphorus uptake by microalgae. While some of the biomass of these samples would have consisted of bacteria it has previously been estimated that the microalgal biomass exceeds the bacterial biomass in terms of volatile suspended solids by 3-5 times (Oron et al., 1979). The samples were kept in the dark in a chilly bin with an ice pack during

transport and were analysed in duplicate within three hours of sampling. This time is not believed to have affected the phosphorus fractions in the sample as it has been shown that microalgal biomass remained stable for approximately one week in dark conditions (Chapter 6).

Table 7.1: Description of full-scale WSP

	Ashhurst WSP	**Aokautere WSP**
Facultative pond dimensions	120 x 220 m	35 x 25 m
Maturation pond dimensions	120 x 60 m	10 x 25 m
Approximate depth	1.5 m	0.7 m (plus approximately 0.5 m of sludge)

Luxury uptake was investigated by determining the phosphorus content in the biomass. This value could then be compared to the 1% phosphorus level that typically occurs in microalgal cells for normal metabolism (Borchardt & Azad, 1968; Kaplan et al., 1986). To determine the phosphorus content, the samples were analysed for total phosphorus and total dissolved phosphorus. The difference between these two measurements was assumed to be the total phosphorus in the biomass, which represents the amount of biological phosphorus uptake. The total phosphorus samples were digested using the nitric acid and sulphuric acid method and analysed using the ascorbic acid assay as described in standard methods (APHA et al., 1995). The biomass concentration was measured as volatile suspended solids (VSS) retained on a Whatman GF/C glass fibre filter. Volatile suspended solids were used to quantify the biomass concentration due to the possible presence of inorganic solids in the field samples.

7.3 Results and discussion

7.3.1 Luxury uptake of phosphorus in full-scale WSP

The phosphorus content in the volatile suspended solids which indicates the degree of luxury uptake was compared and contrasted against the value of 1% which is the typical value for normal microalgal growth (Borchardt & Azad, 1968; Kaplan et al., 1986). The variation in the percentage phosphorus and the occurrence of luxury uptake in the two WSP systems studied is shown in Figure 7.1.

Precipitation of phosphate with cations such as calcium is known to occur at a pH higher than approximately 8.5 (Diaz et al., 1994). The average pH at the time of sampling the ponds was only 7.7 and 7.5 for Ashhurst and Aokautere, respectively. The maximum pH recorded at the time of sampling was 8.5 which only occurred on one occasion. Furthermore, no correlation existed between the pH at the time of sampling and the phosphorus content in the biomass. This implied that during sampling the majority of the phosphorus in the volatile suspended solids was due to biological uptake rather than precipitates.

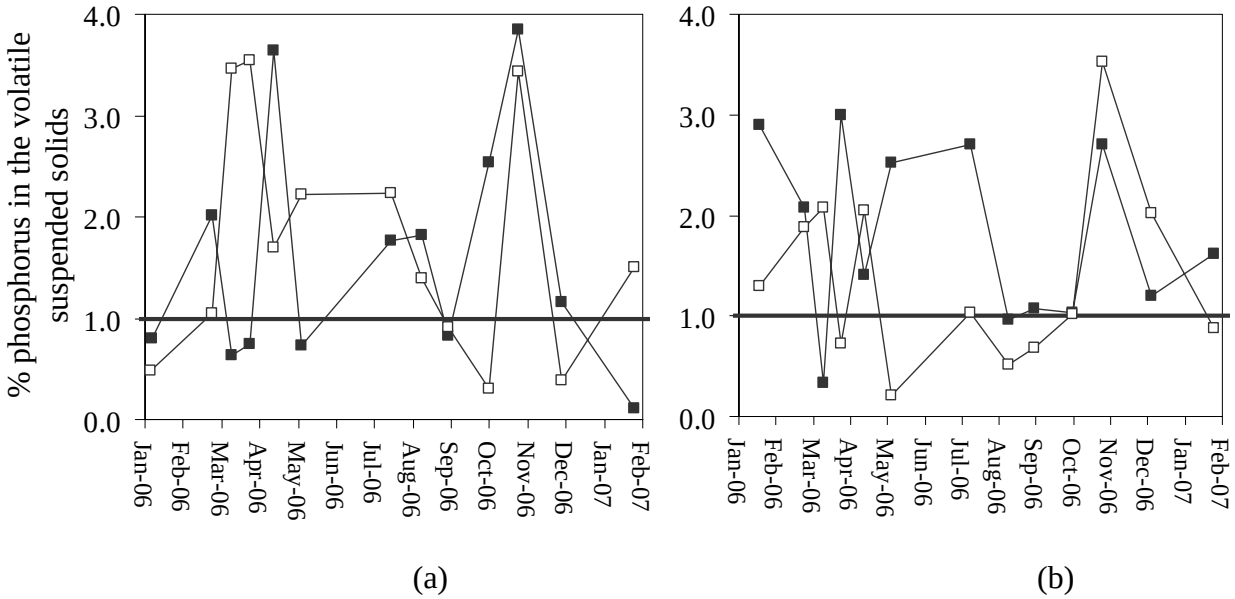

Figure 7.1: Phosphorus content in the volatile suspended solids at Ashhurst (a) and Aokautere (b) WSP for the facultative (■) and maturation ponds (□). The dark line at 1% phosphorus indicates the normal phosphorus content of microalgal biomass.

7.3.1.1 Occurrence of luxury uptake in full-scale WSP

The data in Figure 7.1 shows that the volatile suspended solids in the majority of the samples from both the facultative and maturation ponds contained more than 1% phosphorus and levels of up to 3.85% were recorded. This indicates that luxury uptake of phosphorus did frequently occur in the full-scale WSP studied. This has not previously been reported in the literature.

7.3.1.2 Variation over the year

The data (Figure 7.1) shows that the degree of luxury uptake was variable throughout the year; however, there was no clear seasonal pattern. Additional data such as the temperature and light intensity was also recorded at the time of sampling; however, it was not possible to significantly correlate these measurements to the percentage phosphorus in the volatile suspended solids.

7.3.1.3 Implications for modelling phosphorus removal

These findings are relevant to modelling of phosphorus removal in WSP. Most of the published literature investigating the phosphorus removal mechanisms in WSP has incorrectly assumed that the amount of phosphorus removed by chemical precipitation can be estimated indirectly by measuring the total phosphorus removed and assuming that the biomass contains a fixed portion of phosphorus (for example Bogan et al., 1960; Maiti et al., 1988; Wrigley & Toerien, 1990; Lodi et al., 2003). This assumption is questionable as evidenced by the large variation in the phosphorus content of the biomass as shown in Figure 7.1 and, therefore, many of the earlier estimates of phosphorus removal by precipitation are questionable.

7.3.2 Significance of biological phosphorus uptake

It is important to note that the occurrence of luxury uptake does not necessarily result in high biological phosphorus uptake as this is also dependent on the volatile suspended solids concentration. The phosphorus in the liquid and the phosphorus in the biomass are shown in Figure 7.2.

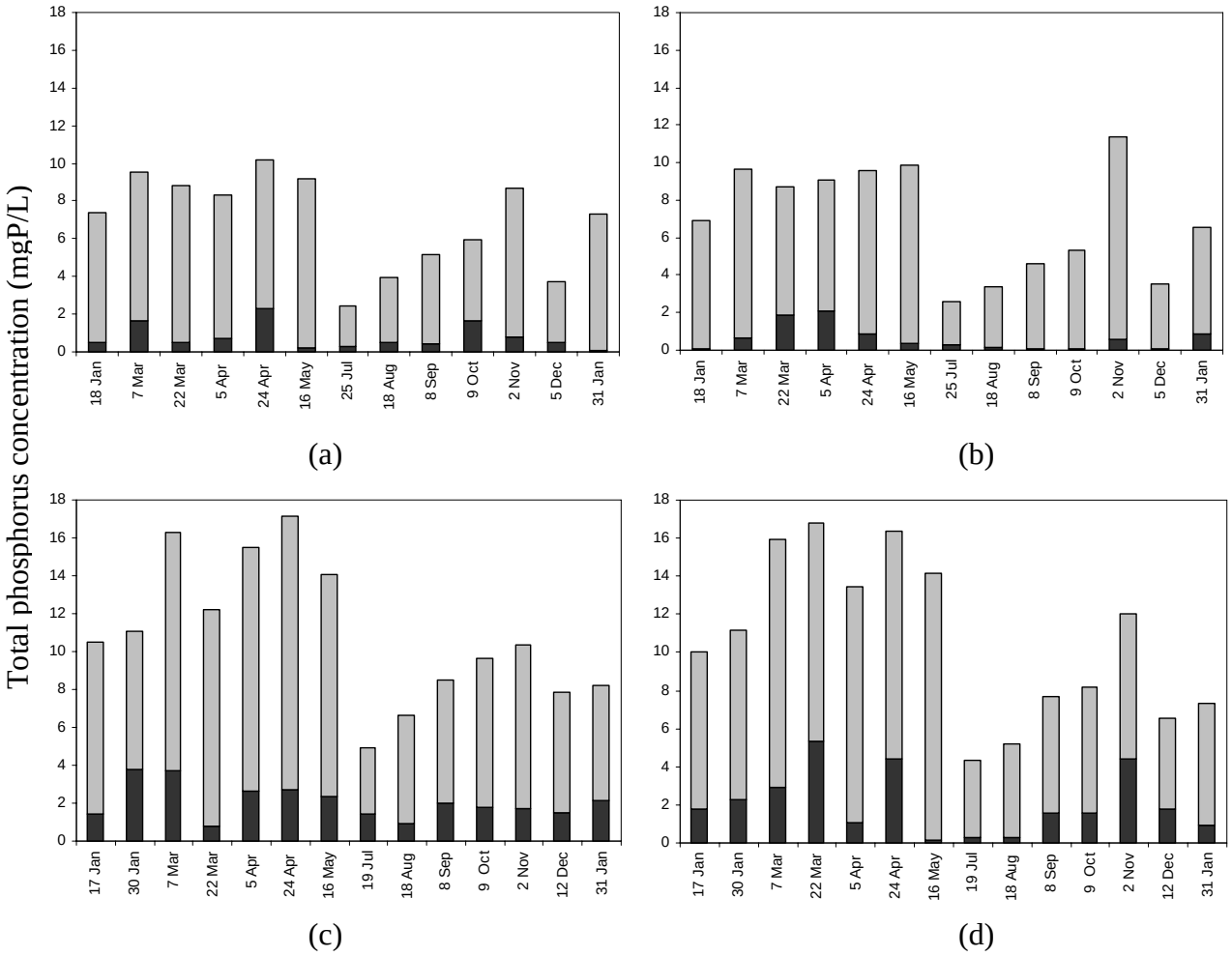

Figure 7.2: Total phosphorus in the liquid (shown in grey) and phosphorus in the volatile suspended solids (shown in black) in the Ashhurst facultative (a) and maturation pond (b) and the Aokautere facultative (c) and maturation pond (d).

7.3.2.1 Variation over the year

Biological phosphorus uptake varies throughout the year as shown by the black section of the bars in Figure 7.2. While some fluctuations would be expected due to changes in the volatile suspended solids concentration, the additional variation as a result of luxury uptake (Figure 7.1) may help to explain the large degree of variation in phosphorus removal often reported in the literature.

Maximum biological phosphorus uptake appears to occur in spring (September – November) and autumn (March – May). Biological uptake is particularly low in winter (June – August) which may be due to a lower light intensity and cooler temperatures depressing the growth rate and thus resulting in a low volatile suspended solids

concentration. However, a reduction in the phosphorus concentration in the liquid (shown by the grey section of the bars in Figure 7.2) could also be responsible for reducing the amount of phosphorus taken up for luxury uptake. This is because luxury uptake has been shown to be reduced when microalgae are exposed to a low phosphorus concentration in the wastewater (Chapter 5). Some of the samples taken during the summer months (December – February) also had low biological uptake. This may be because of photoinhibition during the summer months which limits microalgal growth (Ratchford & Fallowfield, 2002).

7.3.2.2 Limitation to biological phosphorus uptake by microalgae

Luxury uptake has been shown to frequently occur (Figure 7.1) and thus created an expectation that luxury uptake may currently be a significant biological phosphorus uptake mechanism. However, as seen in Figure 7.2 (shown on the previous page) the biological phosphorus uptake (shown by the dark parts of the graph) was relatively small (1–33%) when compared to the total phosphorus in the wastewater sample. In order to understand the implications of this for phosphorus uptake in WSP the data has been reorganised in Figure 7.3 to plot the volatile suspended solids concentration against the degree of luxury uptake (shown by the percentage phosphorus in the biomass).

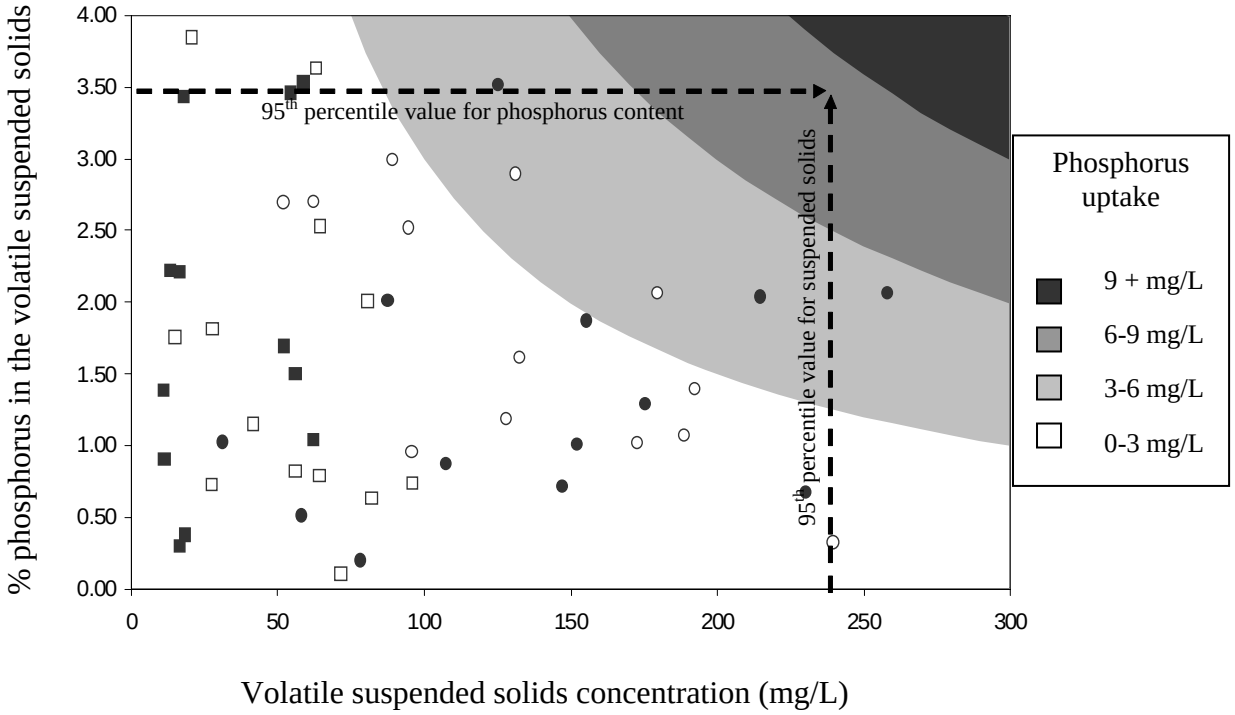

Figure 7.3: Biomass concentration and the phosphorus content of the biomass for each of the WSP sampled (Ashhurst pond 1□, Ashhurst pond 2 ■, Aokautere pond 1 ○, Aokautere pond 2●). The background colours show the net phosphorus uptake calculated for different biomass concentrations and phosphorus content of the biomass. The arrows indicate the 95th percentile values of these parameters.

As can be seen in Figure 7.3 the field data shows that both high volatile suspended solids concentration and luxury uptake can occur but that these parameters do not naturally occur simultaneously. Previous work with batch experiments in the laboratory conducted under controlled conditions provides insight in this regard. These experiments (Chapter 5) showed that accumulation of acid-soluble and acid-insoluble polyphosphate occurred (indicating luxury uptake). However, in periods of high growth the polyphosphate is subsequently utilised by the cells for production of cellular constituents such as phospholipids, nucleic acids and nucleotides. This subsequent transformation from high levels of polyphosphate to high levels of volatile suspended solids means that simultaneously achieving high biomass concentration and high degree of luxury uptake will be rare in field conditions.

7.3.7 Potential of biological uptake as a phosphorus removal mechanism

As discussed above it would appear that the chances of significant amounts of phosphorus removal via luxury uptake in a standard design WSP will always be limited. This is, however, not to say that luxury uptake could not become an important phosphorus removal mechanism with some design modification.

What is of particular significance is that the field data has now shown that high levels of volatile suspended solids and luxury uptake do indeed occur at times in full-scale field WSP. To maximise biological phosphorus uptake both the volatile suspended solids concentration and the phosphorus content in the biomass need to be maximised simultaneously. Several considerations have been proposed in Chapter 5 based on laboratory results. A ‘luxury uptake pond’ has been proposed where concentrated microalgal biomass would be exposed to a high phosphate concentration and high light intensity to maximise polyphosphate accumulation. This ‘luxury uptake pond’ would be mixed preventing settlement of organic solids and thus any subsequent phosphate release from decaying sludge. Biomass harvesting would then be used to remove the polyphosphate rich microalgae from the wastewater. The total phosphorus that could potentially be removed by optimising WSP for luxury uptake and harvesting the biomass is defined by the zone in the top right hand corner of Figure 7.3. For example, if it was possible to achieve and maintain the 95^{th} percentile values of the biomass concentration and luxury uptake, more than 8 mg/L of phosphorus could be uptaken (as shown in the arrows of Figure 7.3). Assuming that all the biomass was removed from the effluent this would result in theoretical biological phosphorus removal in the order of 75–100% based on the average total phosphorus concentration in the wastewater.

7.4 Conclusions

This chapter has shown for the first time that luxury uptake of phosphorus by microalgae does indeed occur in full-scale WSP. In the majority of the samples the biomass was shown to contain more phosphorus than required for normal growth and on one occasion the volatile suspended solids contained almost four times this amount.

This work has shown that variations in biological phosphorus uptake are not simply due to changes in the volatile suspended solids concentration but also due to the variable phosphorus content. This has important implications when modelling phosphorus removal in WSP and shows that some assumptions made in previous publications are questionable.

To improve phosphorus uptake in WSP both the volatile suspended solids concentration and degree of luxury uptake need to be maximised. While high values for volatile suspended solids concentration and phosphorus content of the volatile suspended solids were reported in the field, the maximum values of there two parameters do not naturally occur simultaneously. Previous research has shown that the phosphorus content of the microalgae is dependent on both accumulation and of consumption of phosphorus. During periods of rapid growth the stored phosphorus is rapidly consumed by the biomass. This leads to a high biomass concentration which contains a low phosphorus content.

The results of this chapter have shown that high values of both volatile suspended solids concentration and phosphorus content were at times achieved in full-scale WSP. This therefore provides an opportunity to apply previous laboratory findings to redesign WSP for biological phosphorus removal. Calculations have shown that if the 95th percentile values for the biomass concentration and the phosphorus content of the biomass from the field could be achieved simultaneously 75–100% of the phosphorus in the maturation pond effluent could potentially be removed biologically.

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Chapter 8

Summary

The focus of this thesis was to investigate biological phosphorus removal by microalgae under the conditions typically found in waste stabilisation ponds (WSP). This chapter summarises the main findings of this thesis.

8.1 Initial findings

Screening experiments, reported in Chapter 2, were used to test the influence of a range of environmental factors on biological phosphorus uptake by microalgae. The key environmental factors which were found to influence biological phosphorus uptake were phosphate concentration, light intensity and temperature, while nitrogen concentration in the wastewater and the diurnal light cycle were found to have no significant effect at the levels tested.

The results from the preliminary screening experiments (Chapter 2) also indicated that another biological phosphorus uptake mechanism other than assimilation for microalgal growth was occurring. The findings which supported this were:

- The initial phosphate concentration in the wastewater influenced the amount of phosphorus removed but not microalgal growth.

- Microalgal growth continued to occur even when no phosphate could be detected in the batch reactor.

- The amount of phosphorus that was theoretically required for growth was much lower than the phosphorus uptake measured.

All of these observations suggested that phosphorus was being stored by the microalgae. Luxury uptake, which is the storage of phosphorus in the form of polyphosphate, could explain these findings. While luxury uptake was known to occur in microalgae in natural systems such as lakes, this mechanism had not previously been investigated for WSP.

8.2 The occurrence of luxury uptake

As reported in Chapter 3 luxury uptake was investigated using three techniques that had not previously been used for studying phosphorus removal in WSP. These techniques were microbiological staining, measurement of the percentage phosphorus per unit dry weight, and chemical extraction and measurement of polyphosphate.

The microbiological stain used was specific for polyphosphate granules and showed a large number of polyphosphate granules present in the microalgal cells (Chapter 3, Figure 3.4). However, as staining is not a quantitative technique the percentage phosphorus per unit dry weight was also measured to indicate whether phosphorus was being stored by the biomass. Levels were found to be above 1% phosphorus which indicated that storage of phosphorus was indeed occurring (Borchardt & Azad, 1968; Kaplan et al., 1986). In order to study this mechanism further the different polyphosphate pools in the biomass were also quantified. This was conducted using chemical extraction techniques. This technique enables the separation of acid-soluble polyphosphate (ASP) and acid-insoluble polyphosphate (AISP) which have different roles in metabolism and will be discussed in later sections of this chapter. Analysis of the microalgal biomass showed that both ASP and AISP were present. All three methods therefore indicated that luxury uptake of phosphorus could occur in the microalgae under typical WSP conditions.

8.3 What triggers luxury uptake?

The screening experiments (Chapter 2) showed that biological phosphorus uptake was influenced by phosphate concentration, light intensity and temperature. These key environmental factors were therefore further investigated by analysing the phosphorus content of the biomass using both continuous (Chapter 4) and batch (Chapter 5) reactors. The continuous experiments (Chapter 4) were used to assess the effect of these key environmental factors under continuous culture conditions which are most relevant to the conditions found in full-scale pond systems. However, both the accumulation of polyphosphate and the subsequent consumption need to be considered. Therefore batch experiments, reported in Chapter 5, were used to study the dynamics of the polyphosphate pools and how the key environmental factors influenced these pools with

respect to time. The findings for each of these key environmental factors are discussed below.

8.3.1 Phosphate concentration

The continuous experiments (Chapter 4) showed that while luxury uptake did occur at both of the feed phosphate concentrations tested (5 and 15 mg/L) the phosphate concentration in the feed had no direct effect on luxury uptake. The likely reason for this is due to the well mixed continuous flow configuration of the reactors which meant that the microalgae were exposed to a diluted phosphate concentration within the reactor. While the feed phosphate concentrations tested had no direct effect on luxury uptake there were significant interactions between the phosphate concentration and the other factors tested.

The batch experiments were then used to further investigate the effect of phosphate concentration on the different polyphosphate pools testing a wider range of initial phosphate concentrations (5, 15 and 30 mg/L). ASP was only accumulated in the 15 and 30 mg/L experiments indicating that ASP accumulation was being triggered at a phosphate concentration higher than in the 5mg/L experiment and being definitely observed in the 15 mg/L experiment. The findings of this research indicate that ASP can act as a short term form of phosphorus storage. This has not been previously reported in the literature which is probably because this mechanism had not been studied under WSP conditions. Previous studies have focused on natural systems such as lakes which do not typically have phosphate concentrations high enough for ASP storage to occur.

The batch experiments (Chapter 5) found that AISP accumulation occurred at all phosphate concentrations tested. Consumption of the stored polyphosphate was triggered for the 5 and 15 mg/L experiments (Chapter 5). Once AISP consumption was triggered the AISP decreased rapidly irrespective of whether ASP was present. However, for the 30 mg/L experiment the extracellular phosphate concentration apparently did not drop far enough to reach this trigger point and therefore the AISP was not consumed.

8.3.2 Light intensity

The continuous studies (Chapter 4) showed that the light intensity had a negative effect on luxury uptake due to a decrease in the ASP pool at higher light intensity. This was unexpected as phosphate uptake is known to be an energy requiring process and microalgae receive their energy via photosynthesis. However, further investigation of the dynamics of luxury uptake in the batch experiments explained this finding (Chapter 5). Higher light intensity (150 $\mu E/m^2.s$) resulted in rapid accumulation of ASP but also rapid subsequent consumption for biomass growth. At a lower light intensity (60 $\mu E/m^2.s$) less ASP was initially accumulated, however, the rate of consumption was also much slower. This resulted in the cells at low light intensity retaining more ASP after the initial peak thereby explaining the negative influence observed in the continuous experiments.

Both the continuous (Chapter 4) and batch (Chapter 5) studies showed that while AISP was indeed accumulated at all levels tested, the different levels of light intensity had no significant effect on AISP.

8.3.3 Temperature

The temperature was found to have a positive effect on luxury uptake in the continuous experiments (Chapter 4) which was probably due to an increased rate of accumulation of AISP at a warmer temperature. The batch experiments (Chapter 5) showed that at the warmer temperature (25 °C) a peak in AISP was observed which did not occur at the lower temperature tested (15 °C). This peak in AISP at the warmer temperature was then quickly utilised by the microalgae which resulted in similar levels of AISP being present at both temperatures tested after the initial peak.

While ASP accumulation was shown to occur at all temperatures tested the temperature was found to have no significant effect on ASP in both the continuous (Chapter 4) and batch (Chapter 5) experiments.

8.4 Phosphorus release from sludge

When studying phosphorus removal in WSP it is also important to consider the sludge layer. This is because without biomass harvesting the net amount of phosphorus removal in WSP is determined by the amount of phosphorus that is permanently incorporated into the sludge. Sludge samples were taken from three full-scale WSP and the rate of phosphate release from the WSP sludge samples was determined in the laboratory by measuring the change in phosphate concentration in the overlying liquid over time. The WSP sludge samples from Aokautere and Foxton Beach WSP both had release rates of approximately 4.3 µgP/mgTSS.d. However, the sludge collected from the pond at Ashhurst had a much higher rate of 12.4 µgP/mgTSS.d (Chapter 6). The reason for this difference is likely to be due to an algal bloom occurring at the time that the Ashhurst sludge sample was taken which resulted in a high proportion of fresh microalgal biomass being present in the sludge. The rate of release from fresh laboratory grown microalgal sludge was also tested and was found to be 160 µgP/mgTSS.d. The rate of release from the WSP sludge is comparatively lower than the rate of release from the fresh microalgal sludge simply because the WSP sludge samples would have contained older sludge which would have already been significantly degraded.

Analysis of the different types of phosphorus in the sludge was conducted. It was found that polyphosphate accounted for 33 – 71% of the total phosphorus in the WSP sludge samples (Chapter 6). The fate of polyphosphate in the sludge was also studied as it was previously not known whether polyphosphate would remain in the sludge or whether it was also being released. It had been hypothesised by Kenney et al. (2001) that in lake sediment algal cells could remain intact for many decades and any polyphosphate they contained would not be released. Therefore, the type of phosphorus released from the fresh microalgal biomass was studied in the laboratory (Chapter 6). It was found that polyphosphate was indeed degraded. Other recent research has now also shown that polyphosphate is indeed degraded resulting in phosphate being released from lake sediment (for example Hupfer et al., 2004; Reitzel et al., 2007).

During the release experiments phosphorus was also removed from the wastewater and uptaken by the sludge which was unexpected (Chapter 6). The analysis of the different

phosphorus fractions shows that some polyphosphate was reformed. This reformation of polyphosphate may have contributed to the high levels of polyphosphate measured in the field sludge samples. It is unlikely that this reformation of polyphosphate was due to microalgae unless they were capable of growing heterotrophically as these sludge experiments were conducted in the dark. It might be possible that the phosphorus uptake was due to bacteria as analysis on lake sediments has shown the presence of bacteria that contain polyphosphate granules (Hupfer et al., 1995). It has been suggested that these bacteria may be similar to the bacteria found in enhanced biological phosphorus removal (EBPR) systems (Hupfer et al., 1995; Hupfer et al., 2007; Gloess et al., 2008). These polyphosphate accumulating bacteria require both anaerobic and aerobic conditions for their metabolism and it has been proposed that this may occur in lake sediment due to processes such as resuspension of sediments and microzones near the roots of macrophytes (Hupfer et al., 2007). However, it should be noted that it is most unlikely that these bacteria would have been responsible for polyphosphate accumulation in the microalgal experiments presented in Chapters 2–5 because of the constant highly aerobic conditions that existed in these reactors. It is well documented that constant aerobic conditions do not select for these bacteria (Smolders et al., 1994) and therefore significant growth of polyphosphate accumulating bacteria would not occur in the microalgal reactors.

8.5 Luxury uptake in full-scale WSP

Laboratory based experiments were used to study the luxury uptake mechanism as controlled conditions were required; however, field studies were also undertaken to investigate the occurrence of this mechanism in full-scale WSP under field conditions. The field results discussed in Chapter 7 showed that in the majority of the samples the volatile suspended solids contained more phosphorus than required for growth and that in one instance levels of almost four times this amount was recorded. While luxury uptake of phosphorus by microalgae has been previously identified and investigated in natural ecosystems the findings of this thesis show for the first time that luxury uptake of phosphorus by microalgae does indeed regularly occur in full-scale WSP.

The phosphorus content of the samples from the algal band of the full-scale WSP can be compared to the microalgal culture used in the laboratory. For the field samples the

percentage phosphorus in the volatile suspend solids was 0.21–3.85% (Chapter 7) and in the laboratory the percentage phosphorus was 0.41–3.16% (Chapter 4). This gives confidence that the luxury uptake observed in the laboratory work is indicative of what is occurring in full-scale WSP.

The variability in the phosphorus content of the microalgae has implications when modelling phosphorus removal in WSP systems. Several authors when modelling phosphorus removal in WSP have determined biological phosphorus removal by assuming a fixed portion of phosphorus in the biomass. However, the findings from the full-scale WSP presented in Chapter 7 shows that the phosphorus content of the volatile suspended solids is actually highly variable which indicates that this assumption made by previous researchers is questionable.

Phosphorus removal from full-scale WSP has often been reported as being highly variable in the literature. While fluctuations in microalgal growth would be expected to cause some variation in phosphorus removal, this additional variation in the phosphorus content as a result of luxury uptake helps to explain the large degree of variation reported in full-scale WSP.

To achieve high levels of biological uptake both the biomass concentration and the phosphorus content of the biomass need to be maximised simultaneously. However, the field results indicate that maximum volatile suspended solids concentration and phosphorus content do not naturally occur simultaneously in the field. This is to be expected because, as discussed in Chapter 5, during high biomass growth polyphosphate is utilised. However, what is of particular significance is that the field data has now shown that high levels of volatile suspended solids and phosphorus content do indeed occur at times in full-scale field WSP. This means that based on the laboratory findings it may be possible to improve phosphorus removal in full scale field systems. This is further discussed in a later section of this chapter.

8.6 An overview of biological phosphorus removal in WSP

Figure 8.1 gives an overview of the biological phosphorus removal mechanisms in WSP. Phosphorus enters the WSP as a component of the influent wastewater. As shown in Figure 8.1 there are then several different mechanisms that can result in phosphorus moving between the different phosphorus pools. The three main pools of phosphorus are shown in bold as being the phosphorus in the wastewater, the phosphorus in the biomass and the phosphorus in the sludge (Figure 8.1). The mechanisms responsible for the movement of phosphorus between these three pools are identified and the key environmental factors that have been shown to influence these processes have been indicated in italics in Figure 8.1.

Phosphorus is assimilated by microorganisms for growth as it is required for the synthesis of cellular constituents. In addition to growth, microalgae in WSP have now been shown to take up phosphorus for luxury uptake. Biological phosphorus uptake, which includes both growth and luxury uptake, has been shown to be influenced by the phosphate concentration, light intensity and temperature (Chapter 2). The movement of phosphorus between the different phosphorus pools within the biomass is based on the findings of Miyachi et al. (1964) while the key environmental factors which influence this movement have been defined by this thesis.

As the microorganisms die the biomass settles into the sludge layer. Some of this phosphorus including the polyphosphate accumulated via luxury uptake is degraded and subsequently phosphate is released back into the overlying wastewater, although as indicated in Figure 8.1, it also appears that some phosphate is taken up by the microorganisms in the sludge.

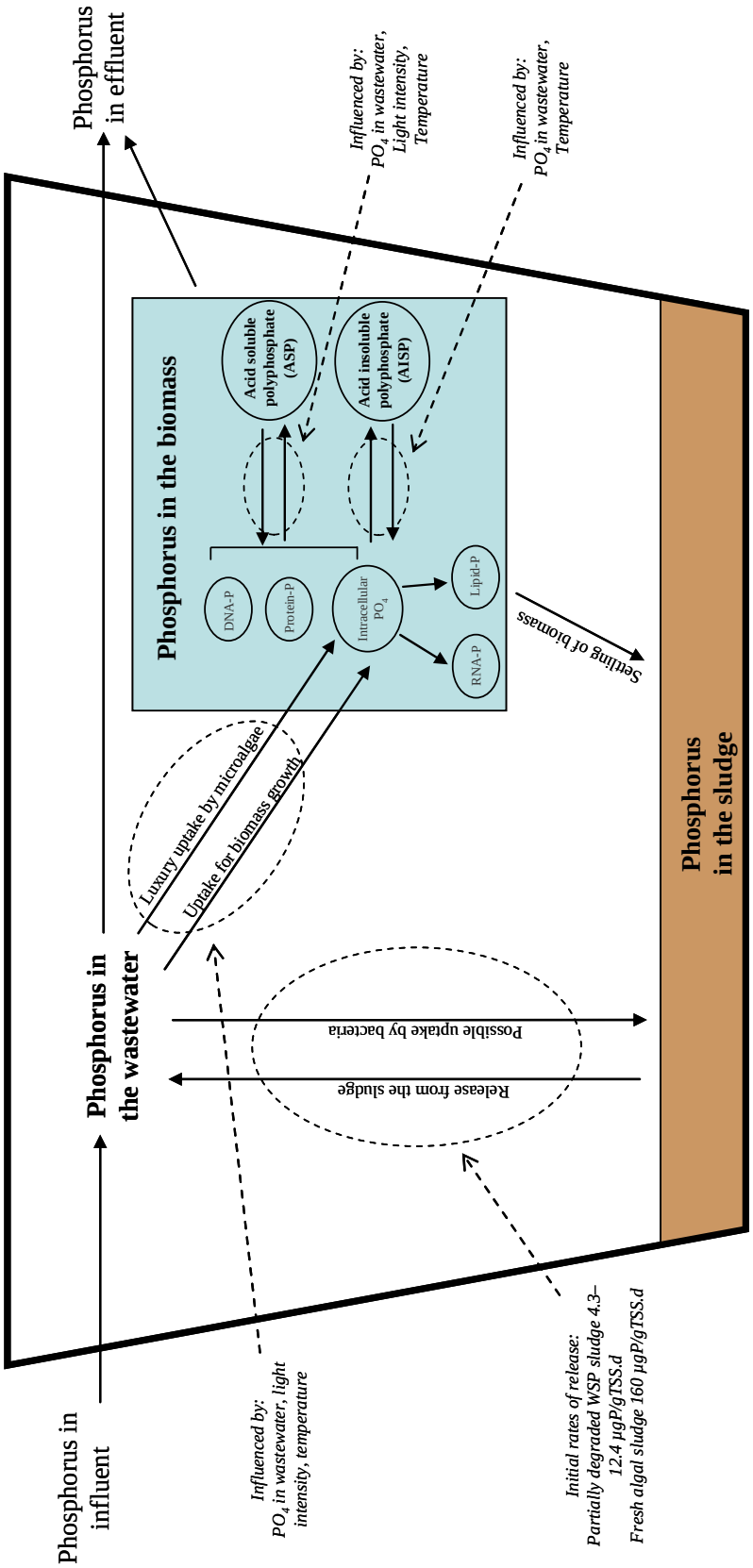

Figure 8.1: Summary of the biological phosphorus removal mechanisms in WSP. Note that for clarity chemical mechanisms such as precipitation and physical mechanisms such as primary sedimentation of solids are not included.

8.7 Developing a new phosphorus removal process

With this improved understanding of the biological phosphorus uptake via luxury uptake and the key environmental factors which influence this mechanism it may be possible to develop a new process to optimise phosphorus removal via luxury uptake by microalgae.

A number of proposals have been developed from this research that could be considered when developing such a process (as discussed in Chapter 5). These include ideas such as maximising the biomass concentration, exposing the microalgae to a high phosphate concentration, optimising light levels and biomass harvesting to remove the microalgae rich in polyphosphate so that phosphorus is removed from the system. To expose the microalgae to the required conditions a ‘luxury uptake pond’ is proposed. The wastewater from the ‘luxury uptake pond’ could then undergo further treatment in the main pond system to ensure adequate pathogen removal and to further polish the effluent. It should be noted that these proposals are based on laboratory findings and therefore further trials to refine this process would be needed before this process could be scaled up.

8.8 Further research

The synthetic wastewater used in this thesis did not contain readily degradable carbon in order to limit bacterial growth and allow biological phosphorus removal by microalgae to be studied, which was the objective of this thesis. The findings of this research would apply to low carbon environments which are typical of maturation ponds. Further research would be needed to investigate whether increased bacterial growth in the presence of organic carbon, would influence biological phosphorus uptake by microalgae. Perhaps the most obvious effect is that a higher concentration of bacteria would influence microalgal growth by reducing light penetration. Furthermore, an increase in bacterial growth is likely to result in larger microalgal-bacterial flocs being formed which may also reduce the light intensity that the microalgae are exposed to. These effects are important as light intensity was shown to be one of the key factors influencing luxury uptake. In addition to the effects of bacterial growth on light intensity it is also known that bacteria can produce both growth promoting (de-Bashan

et al., 2004) and growth limiting substances (Rhee, 1972). It may therefore be possible that bacteria produce extracellular substances which influence phosphorus uptake by microalgae. However, the effects that increased bacterial growth could have on microalgal phosphorus uptake have not yet been investigated in pond systems or the natural environment.

The laboratory findings of this thesis have been used to propose a ‘luxury uptake pond’ where the microalgae are exposed to the conditions which trigger luxury uptake. Further laboratory research is required to test this proposed system and refinement such as determining the optimal retention time of the pond in order to maximise polyphosphate accumulation while minimising subsequent polyphosphate consumption would also be needed. The next step would be to test the ‘luxury uptake pond’ at pilot scale to test this system under field conditions and allow the process engineering of the system to be developed. For example the ‘luxury uptake pond’ will need to be plug flow to allow the microalgae to be exposed to a high initial phosphate concentration. The mixing of the system will also be important as a high biomass concentration is needed to achieve high phosphorus uptake, however; the high biomass concentration will lead to mutual shading which will decrease the light penetration. Therefore, vertical mixing of the system will be very important to ensure that the microalgae are exposed to enough sunlight.

The results presented in Chapter 6 of this thesis suggest that bacteria in the sludge can assimilate phosphorus and store this phosphorus as polyphosphate. While similar findings have been reported in lake sediment, the mechanism occurring and the type bacteria responsible has not been determined. It has been suggested that these bacteria are similar to those found in enhanced biological phosphorus removal systems and that the aerobic and anaerobic conditions which are needed for the metabolism of these bacteria might occur due to resuspension of sediment (Hupfer et al., 2007), however this has not yet been confirmed. It may be possible that if the mechanisms occurring and the factors which influence this process were understood a new process could be developed to enhance phosphorus removal in WSP utilising bacteria.

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