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# Towards a luxury uptake process via microalgae – Defining the polyphosphate dynamics

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ARTICLE INFO

*Article history:*
Received 24 November 2008
Received in revised form
21 May 2009
Accepted 1 June 2009
Published online 11 June 2009

*Keywords:*
Luxury uptake
Microalgae
Nutrient removal
Phosphorus
Polyphosphate
Waste stabilization ponds

ABSTRACT

Microalgae in waste stabilization 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 utilizing luxury uptake by microalgae might be developed. In this work, luxury uptake was investigated by chemical extraction of the acid-soluble and acid-insoluble fractions of polyphosphate in the microalgae. The results showed that the initial accumulation and subsequent utilization 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 utilization 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 show that ASP can also act as a short term form of phosphorus storage. To optimize 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.

## 1. Introduction

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. WSP provide effective wastewater treatment in terms of organic carbon and pathogen removal. However, phosphorus removal 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

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doi:10.1016/j.watres.2009.06.011

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 utilizes luxury uptake of phosphate by bacterial biomass. However, compared to WSP, EBPR requires relatively large amounts of energy, requires ongoing 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 an essential element that is 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 and Azad, 1968; Kaplan et al., 1986), but 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 concentration of phosphorus is limiting (Kuhl, 1974).

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 and 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 does not require 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 and Azad, 1968; Aitchison and Butt, 1973; Gotham and Rhee, 1981; Jansson, 1993) the factors which influence luxury uptake are poorly understood. Apart from Powell et al. (2008), luxury uptake by microalgae has not been previously studied under the conditions found in WSP and has otherwise been totally overlooked in regard to its potential as a phosphorus removal technique for WSP.

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 utilized by the cell when the external phosphorus concentration is limiting for growth (Miyachi and Miyachi, 1961; Miyachi and 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 and 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 and so are poorly understood. In order to progress towards the development of a new phosphorus removal process the dynamics of the luxury uptake mechanism must be studied.

This paper investigates the dynamics of the luxury uptake mechanism by making direct measurements of the polyphosphate in the microalgae using chemical extraction techniques. The work investigates the effect of phosphate concentration, light intensity and temperature on the polyphosphate concentration in the microalgae with respect to time. While understanding already exists of how phosphorus moves between the intracellular pools this work will help to define how these factors influence the dynamics of these transformations. The results of the study then allow possible methods of optimizing for phosphorus removal via luxury uptake by microalgae in full-scale pond systems to be proposed.

## 2. Materials and methods

### 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, 36 W) 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 and LOGGER 2100 software; 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 as shown in Table 1. The levels of these factors tested were selected for their relevance to those typically found in full-scale WSP.

### 2.2. Inoculum

A continuous-flow reactor, initially inoculated with water from a WSP and fed with synthetic wastewater (Davis and Wilcomb, 1967), was used to provide a consistent source of inoculum for the batch experiments. This inoculum reactor had a hydraulic retention time of 10 days and it contained a mixed culture dominated by the microalga *Scenedesmus*. This reactor was allowed to reach steady-state prior to use as an inoculation source for the batch experiments. Further details of this continuous-flow inoculum reactor can be found in Powell et al. (2006).

The phosphorus content of this culture has previously been analyzed under a range of different environmental conditions and was shown to contain 0.41–3.16% phosphorus (Powell et al., 2008). This is similar to the phosphorus content of microalgal samples taken from full-scale WSP systems over a 12-month period (0.21–3.85% as discussed in Powell, 2009) which gives confidence that this culture is representative of the microalgae found in full-scale WSP.

### 2.3. Synthetic wastewater

Synthetic wastewater was used to ensure a consistent composition for all experiments. The synthetic wastewater (Davis and Wilcomb, 1967) contained the metal ion chelating additives EDTA (ethylenediaminetetraacetate) and sodium citrate to prevent possible removal of phosphorus by precipitation so that the biological removal mechanisms could be studied. 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.

### 2.4. Analytical analysis

The phosphate concentration was measured using ion chromatography (Dionex ICS-2000; Dionex Corporation, Sunnyvale, CA, USA) as described previously (Powell et al., 2008). The microalgae was measured as dry weight using a 0.45 μm filter membrane (Lee and Shen, 2004).

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 analyzed for total phosphorus to determine the ASP and AISP. Total phosphorus samples were analyzed using the sulphuric acid and nitric acid digestion followed by the ascorbic acid colorimetric method in accordance with standard methods (APHA et al., 1995).

### 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 the statistical software 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.

**Table 1 – Experimental matrix.**

| Experiment | Phosphorus (mg P/L) | Light intensity ($\mu E/m^2 s$) | 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 |
| I | 30 | 150 | 25 |

## 3. Results and discussion

### 3.1. Effect of phosphate concentration

The phosphate concentrations tested in these experiments were 5, 15 and 30 mg/L although the actual starting phosphate concentrations (as shown in Fig. 1) were slightly lower than these values because of dilution from the inoculum which contained a lower phosphate concentration.

As shown in Fig. 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 (Fig. 1a), 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 and Miyachi, 1961; Miyachi and Tamiya, 1961; Miyachi et al., 1964) which enables several cell divisions in the absence of any external phosphate (Jansson, 1988; John and Flynn, 2000).

At higher initial phosphate concentrations of 15 and 30 mg/L (Fig. 1b and c) accumulation of both ASP and AISP occurred. These results (Fig. 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.

Once the phosphate in the wastewater had been consumed the polyphosphate in the microalgae decreased rapidly (Fig. 1a and b) as the cells used the accumulated polyphosphate for growth. However, as can be seen in Fig. 1c at the highest level of phosphate tested (30 mg/L), polyphosphate was accumulated and only partially consumed by the cells. In fact, Fig. 1c shows that after the initial AISP peak there was little change in the AISP concentration. 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 and Miyachi, 1961; Miyachi and Tamiya, 1961; Miyachi et al., 1964). As can be seen in Fig. 1c, phosphate was present in the wastewater throughout the experiment which would explain why the AISP was not utilized. Because the rate of

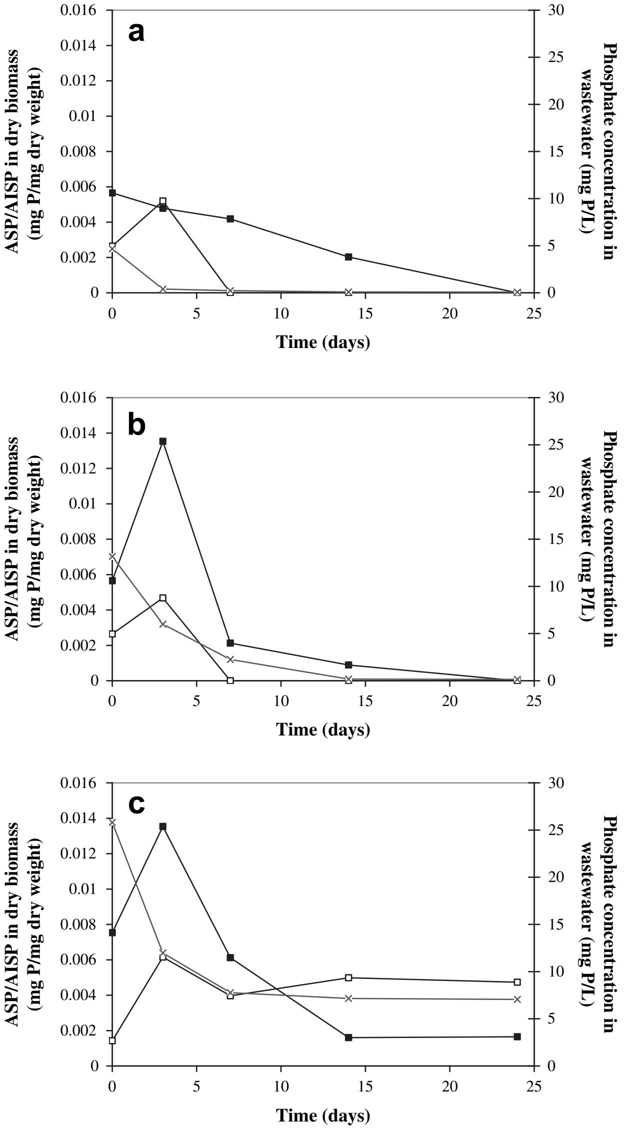

**Fig. 1 – Initial phosphate concentration of 5 mg P/L (a), 15 mg P/L (b) and 30 mg P/L (c). ASP per unit dry weight (■), AISP per unit dry weight (□) and phosphate concentration in the wastewater (×).**

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 utilized as a phosphorus source for growth.

While ASP is known to be involved in metabolism the results of this study show that ASP can also act as a form of short term polyphosphate storage as evidenced by the peaks in Fig. 1b and c. However, while AISP could be stored over a long period of time as shown in Fig. 1c, ASP was rapidly utilized after approximately three days (Fig. 1).

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.

### 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). Fig. 2a shows the effect of temperature on the ASP in the microalgae.

As might be expected the temperature has some influence on both the accumulation and subsequent consumption of the ASP (Fig. 2a). A key difference that can be seen in Fig. 2a is that the ASP is consumed more rapidly at the higher temperature (25 °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 Fig. 2b temperature 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

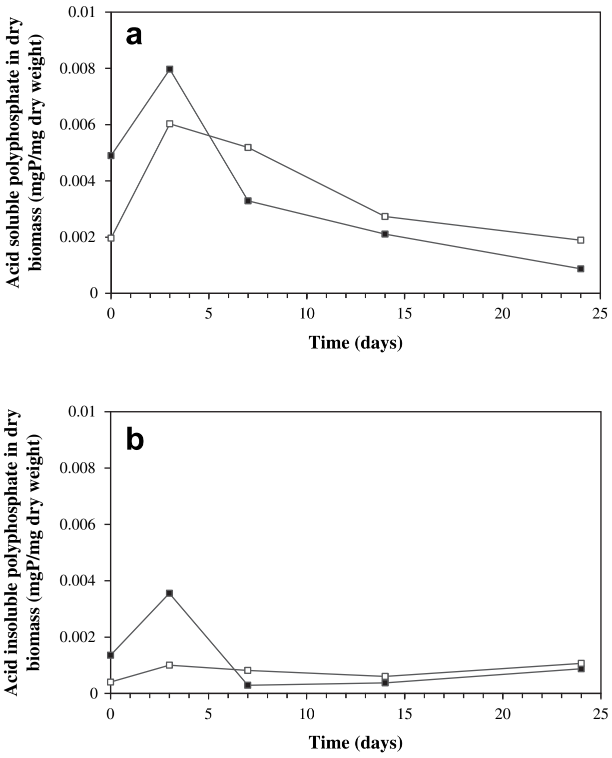

**Fig. 2 – 'Main effects plot' of the influence of temperature (25 °C ■, 15 °C □) on the ASP per unit dry weight (a) and AISP per unit dry weight (b).**

both daily and seasonally. Although polyphosphate accumulation was influenced by temperature, significant quantities of polyphosphate were accumulated at both of the temperatures tested which suggests that this removal mechanism will operate under a variety of climatic conditions.

### 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 have assessed the influence of different light intensities. The effect of light intensity on the AISP content of the microalgae is shown in Fig. 3b.

Light intensity was shown to not have any significant effect (*p*-value 0.466) on the AISP in the microalgae. Fig. 3a 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 (Fig. 3a). While Fig. 3a 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) (Fig. 3a).

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 optimize phosphate removal. 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 depends on their 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 optimizing 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 optimized. As shown in Fig. 3a, 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.

### 3.4. *Summary of findings*

The two main outcomes of this research 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 techniques that could lead to development of a process utilizing luxury uptake by microalgae can be proposed.

#### 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 utilization in microalgae very complex. In Fig. 4 the findings reported in this paper have been summarized to illustrate the transformations that occur between the different phosphorus pools and the factors that affect these transformations. While the biological transformations in Fig. 4 are based on previous literature it is the work presented in this paper 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.

The AISP pool is used by the microalgae as a phosphorus store (Miyachi et al., 1964). This polyphosphate is only utilized when the wastewater phosphate concentration reaches a growth limiting level. Our findings show that the AISP in the microalgae depends on the temperature and the phosphate concentration in the wastewater (Fig. 4).

As illustrated in Fig. 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).

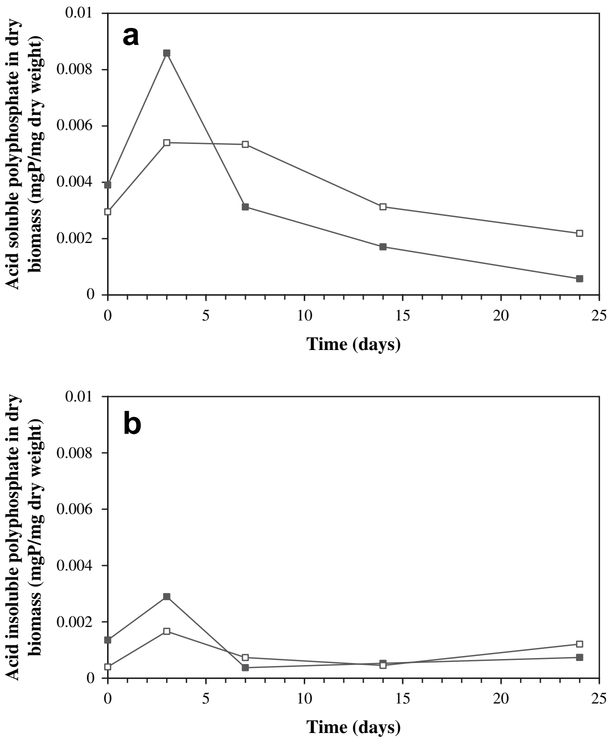

**Fig. 3 – 'Main effects plot' of the influence of light intensity (150 μE/m$^2$ s ■, 60 μE/m$^2$ s □) on the ASP per unit dry weight (a) and AISP per unit dry weight (b).**

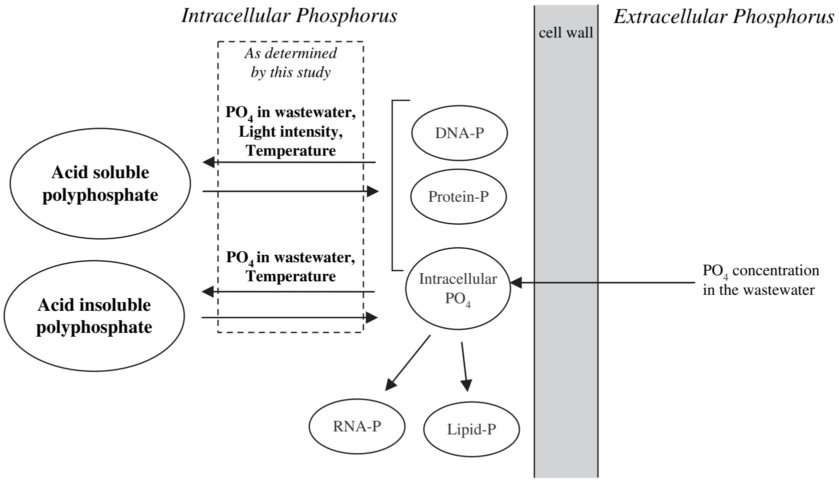

**Fig. 4 – Summary diagram of how factors affect the polyphosphate pools in WSP microalgae. Transformations between pools are adapted from Miyachi et al. (1964).**

Our findings have shown that the ASP in the microalgae is dependent on 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 paper show 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.

### 3.4.2. *Towards a phosphorus removal process*

The relatively low microalgal concentration typically found in WSP means that biological phosphorus removal in WSP is generally low. However, if luxury uptake is triggered phosphorus uptake from the wastewater could be more than tripled as discussed in Powell et al. (2008). Additionally if the microalgal suspended solids concentration was increased or concentrated, very high levels of biological phosphorus uptake might then be achieved.

While previous research had determined the key factors that influence luxury uptake (Powell et al., 2008) the dynamics of the luxury uptake mechanism studied in this paper now enables the environmental conditions required to trigger luxury uptake to be proposed. This is an important first 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 optimize 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, maximizes polyphosphate accumulation (Fig. 1).
- The experimental results (Fig. 3a) 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 minimized yield of biomass with a maximized phosphorus concentration.

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, continuous-flow reactors.

## 4. Conclusions

The accumulation and utilization of both ASP and AISP were found to be a function of the phosphate concentration in the wastewater. Light intensity influenced both the accumulation and utilization of ASP. At higher light intensity the initial accumulation of ASP was higher; however, the ASP was then rapidly consumed and ultimately resulted in higher ASP 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 show 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 optimized 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 minimized yield of biomass with a maximized phosphorus concentration. The final step would be to harvest the phosphorus rich microalgae.

## Acknowledgements

Mr Chris Pepper and the Palmerston North City Council are acknowledged for supporting this project.

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