

# LUXURY UPTAKE OF PHOSPHORUS BY MICROALGAE IN FULL-SCALE WASTE STABILISATION PONDS

*N. Powell*<sup>1</sup>, *A. Shilton*<sup>1</sup>, *S. Pratt*<sup>2</sup>, *Y. Chisti*<sup>1</sup>

<sup>1</sup>*School of Engineering and Advanced Technology, Massey University, Palmerston North, New Zealand*

<sup>2</sup>*Advanced Water Management Centre, University of Queensland, St Lucia Campus, Australia*

---

## ABSTRACT

Biological phosphorus removal 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 it 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.

## KEYWORDS

**Luxury uptake; phosphorus removal; polyphosphate; waste stabilisation ponds**

## 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<sup>th</sup> and 95<sup>th</sup> 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 removal by microalgae is largely thought to be due to its uptake for normal growth. However, removal 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 (Powell et al., 2008; Powell et al., 2009). However, the question remains whether such a phenomenon is present in full-scale field WSP and if so to what extent.

This paper 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.

## 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 1. In each case both the facultative and maturation ponds were sampled. The 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 removal by microalgae.

*Table 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 retained on a Whatman GF/C glass fibre filter.

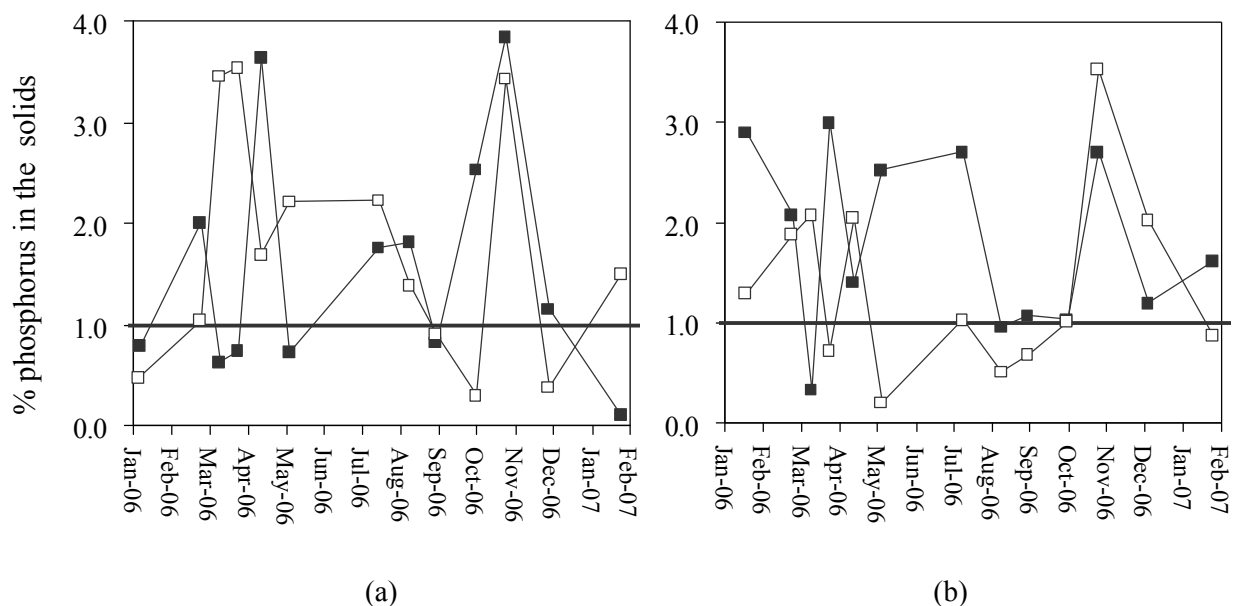
## 3 RESULTS AND DISCUSSION

### 3.1 LUXURY UPTAKE OF PHOSPHORUS IN FULL-SCALE WSP

The phosphorus content in the biomass 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 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 solids was due to biological uptake rather than precipitates.

Figure 1: Phosphorus content in the biomass 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.



#### 3.1.1 OCCURRENCE OF LUXURY UPTAKE IN FULL-SCALE WSP

The data in Figure 1 shows that in the majority of the samples the biomass 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.

#### 3.1.2 VARIATION OVER THE YEAR

The data (Figure 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 biomass.

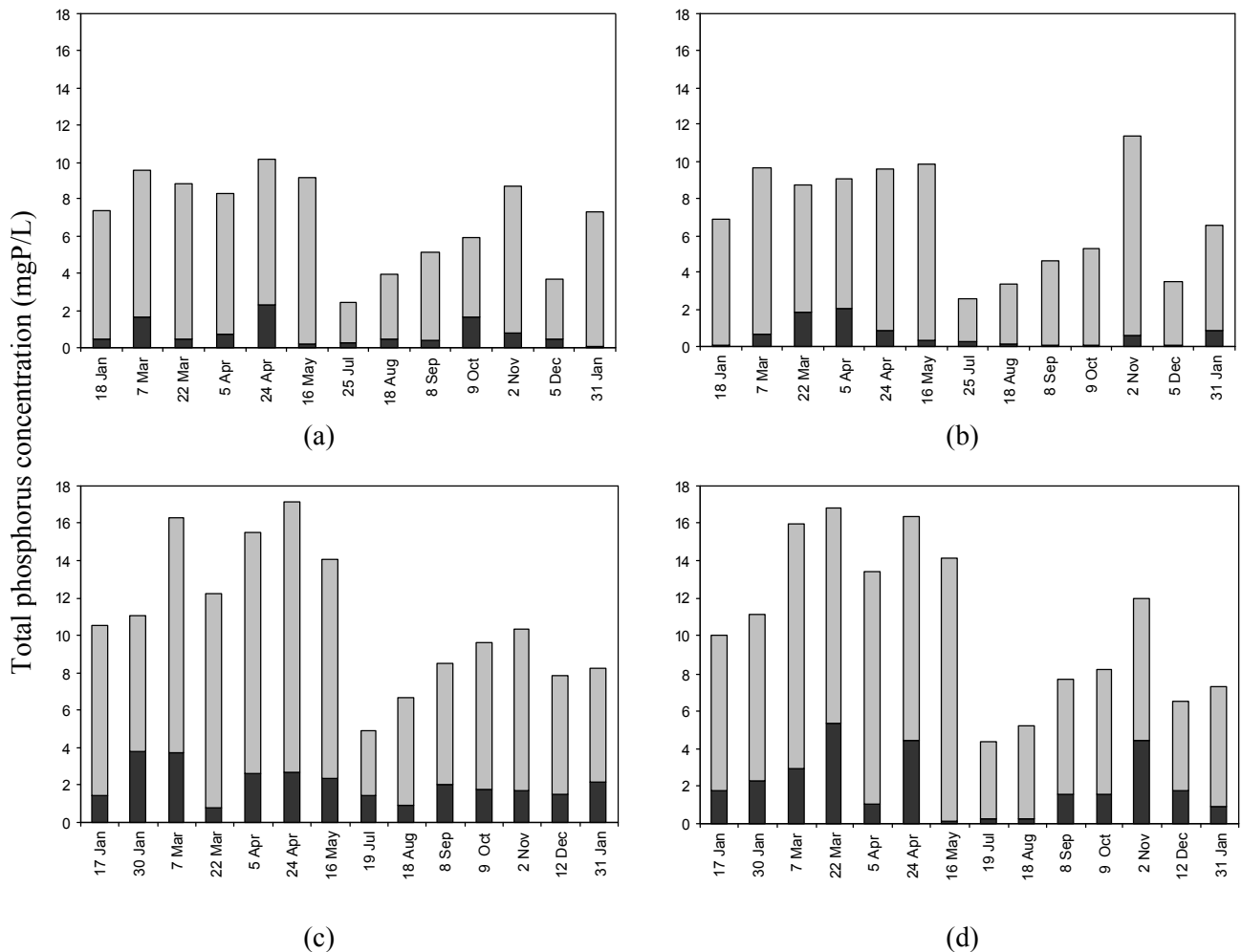
### 3.1.3 IMPLICATIONS FOR MODELING PHOSPHORUS REMOVAL

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 1 and, therefore, many of the earlier estimates of phosphorus removal by precipitation are questionable.

### 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 uptake is also dependent on the biomass concentration. The phosphorus in the liquid and the phosphorus in the biomass are shown in Figure 2.

Figure 2: Total phosphorus in the liquid (shown in grey) and the biomass (shown in black) in the Ashhurst facultative (a) and maturation pond (b) and the Aokautere facultative (c) and maturation pond (d).



#### 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 2. While some fluctuations would be expected due to changes in the biomass concentration, the additional

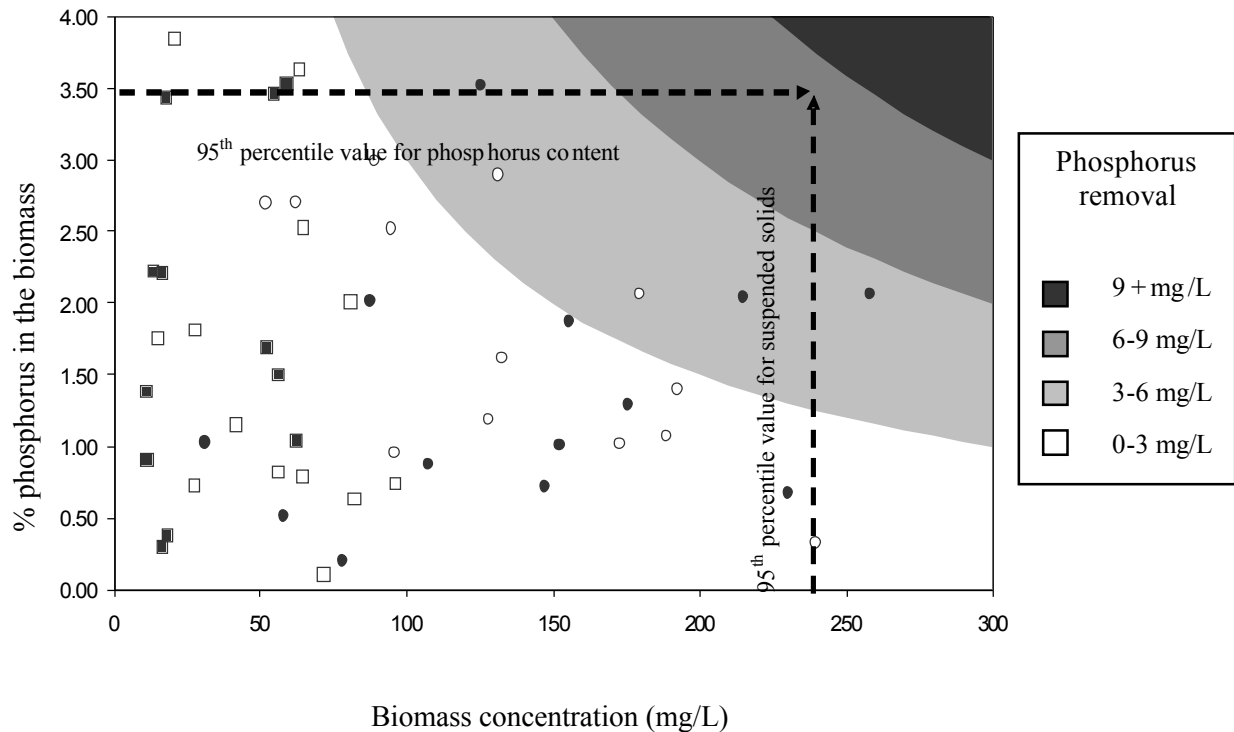
variation as a result of luxury uptake (Figure 1) may help to explain the large degree of variation in phosphorus removal often reported in the literature.

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 biomass concentration. However, a reduction in the phosphorus concentration in the liquid (shown by the grey section of the bars in Figure 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 (Powell et al., 2009). 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).

### 3.2.2 LIMITATION TO BIOLOGICAL PHOSPHORUS UPTAKE BY MICROALGAE

Luxury uptake has been shown to frequently occur (Figure 1) and thus created an expectation that luxury uptake may currently be a significant biological phosphorus uptake mechanism. However, as seen in Figure 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 sample. In order to understand the implications of this for phosphorus uptake in WSP the data has been reorganised in Figure 3 to plot the biomass concentration against the degree of luxury uptake (shown by the percentage phosphorus in the biomass).

Figure 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 at the WSP studied.



As can be seen in Figure 3 the field data shows that both high biomass 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. The results from Powell et al. (2009) 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 biomass means that simultaneously achieving high biomass concentration and high degree of luxury uptake will be rare in field conditions.

### **3.2.3 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 biomass concentration and luxury uptake do indeed occur at times in full-scale field WSP. However, to maximise biological phosphorus removal both the biomass concentration and the phosphorus content in the biomass need to be maximised simultaneously. Several considerations have been proposed in Powell et al. (2009) 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. 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 3. For example, if it was possible to achieve and maintain the 95<sup>th</sup> percentile values of the biomass concentration and luxury uptake, approximately 8 mg/L of phosphorus could be uptaken (as shown in the arrows of Figure 3) which would result in possible biological phosphorus removal in the order of 75–100% based on the average total phosphorus concentration in the wastewater.

## **4 CONCLUSIONS**

This paper 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 biomass contained almost four times this amount.

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

To improve phosphorus removal in WSP both the biomass concentration and degree of luxury uptake need to be maximised. While high values for biomass concentration and phosphorus content of the biomass were reported in the field, the maximum values of these 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 paper have shown that high values of both biomass 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 95<sup>th</sup> 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.

## **ACKNOWLEDGEMENTS**

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

## REFERENCES

- APHA, AWWA and WEF. (1995) *Standard methods for the examination of water and wastewater*, 19th ed., Washington, D.C., Water Environment Federation.
- Bento, A. P., Ribeiro, L. F., Sartorato, J. and Lapolli, F. R. (2002) 'Wastewater treatment using stabilisation ponds: Florianopolis experience, south of Brazil', 5th International IWA Specialist Group Conference on Waste Stabilisation Ponds: Pond Technology for the New Millennium.
- Bogan, R. H., Albertson, O. E. and Pluntze, J. C. (1960) 'Use of algae in removing phosphorus from sewage' *Journal of the Sanitary Engineering Division: Proceedings of the American Society of Civil Engineers*, 86, 5, 1-20.
- Borchardt, J. A. and Azad, H. S. (1968) 'Biological extraction of nutrients' *Journal of Water Pollution Control Federation*, 40, 10, 1739-1754.
- Davies-Colley, R. J., Hickey, C. W. and Quinn, J. M. (1995) 'Organic matter, nutrients, and optical characteristics of sewage lagoon effluents' *New Zealand Journal of Marine and Freshwater Research*, 29, 2, 235-250.
- Diaz, O. A., Reddy, K. R. and Moore, P. A. J. (1994) 'Solubility of inorganic phosphorus in stream water as influenced by pH and calcium concentration' *Water Research*, 28, 8, 1755-1763.
- Kaplan, D., Richmond, A. E., Dubinsky, Z. and Aaronson, S. (1986) Algal nutrition, In A. E. Richmond (Ed.), *CRC handbook of microalgal mass culture*, Boca Raton, CRC Press.
- Lodi, A., Binaghi, L., Solisio, C., Converti, A. and Del Borghi, M. (2003) 'Nitrate and phosphate removal by *Spirulina platensis*' *Journal of Industrial Microbiology & Biotechnology*, 30, 11, 656-660.
- Maiti, S. K., Gupta, S. K. and Joshi, S. G. (1988) 'Nutrients removal and conservation by activated algae in oxidation ditch' *Journal of Water Pollution Control Federation*, 60, 12, 2115-2119.
- Moutin, T., Gal, J. Y., El Halouani, H., Picot, B. and Bontoux, J. (1992) 'Decrease of phosphate concentration in a high rate pond by precipitation of calcium phosphate: Theoretical and experimental results' *Water Research*, 26, 11, 1445-1450.
- Nurdogan, Y. and Oswald, W. J. (1995) 'Enhanced nutrient removal in high-rate ponds' *Water Science and Technology*, 31, 12, 33-43.
- Pearson, H. (2005) Microbiology of waste stabilisation ponds, In A. Shilton (Ed.), *Pond Treatment Technology*, London, IWA Publishing.
- Powell, N., Shilton, A., Chisti, Y. and Pratt, S. (2009) 'Towards luxury uptake process via microalgae - Defining the polyphosphate dynamics' *Water Research*, doi:10.1016/j.watres.2009.1006.1011.
- Powell, N., Shilton, A., Pratt, S. and Chisti, Y. (2008) 'Factors influencing luxury uptake of phosphorus by microalgae in waste stabilization ponds' *Environmental Science and Technology*, 42, 16, 5958-5962.
- Ratchford, I. A. J. and Fallowfield, H. J. (2002) 'The effect of light:dark cycles of medium frequency on photosynthesis by *Chlorella vulgaris* and the implications for waste stabilisation pond design and performance', 5th International IWA Specialist Group Conference on Waste Stabilisation Ponds: Pond Technology for the New Millennium, 2-5 April.
- Wrigley, T. J. and Toerien, D. F. (1990) 'Limnological aspects of small sewage ponds' *Water Research*, 24, 1, 83-90.