Elevating phosphorus accumulation in waste stabilisation pond algae

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Abstract

Facultative waste stabilisation ponds (WSP) are used globally for wastewater treatment due to their low cost and simple operation. While WSPs can be effective at removing organic pollutants and pathogens, phosphorus removal is typically poor. Algae that are common in WSPs are known to accumulate phosphorus and increase their phosphorus content in the biomass from 1% up to 3.8% (gP/gSS), which is believed to be from the production of intracellular polyphosphate granules. This phenomenon, known as luxury uptake, may be possible to manipulate to improve phosphorus removal in WSPs; however, its occurrence is sporadic and poorly understood. This PhD thesis was undertaken to investigate the conditions that influence phosphorus accumulation in WSP algae. Phosphorus accumulation was quantified using two methods: (1) the traditional phosphorus content in the biomass (gP/gSS), and (2) a new image analysis method developed in this thesis that quantifies stained polyphosphate granules within individual algal cells (μm^2 granule/μm^2 cell).

Following a literature review and screening experiments that sought to identify variables that could affect the phosphorus content in the biomass (gP/gSS), six variables: temperature, phosphorus concentration, light intensity, mixing intensity, organic load, and pH were comprehensively examined using 40 batch factorial experiments (2^{6-1}) and a mixed genus culture from a full-scale WSP. Nine variables and interactions had a significant effect on the phosphorus content in the biomass and were incorporated into a regression equation. This ‘mixed genus’ regression equation was tested against literature data, where seven out of the eight batch experiments from the literature were successfully predicted.

In order to identify if the batch findings could be applied to a continuous process, which is more typical of full-scale WSPs, a bench-scale novel ‘luxury uptake’ process was designed, built, and operated under five different scenarios. The regression equation successfully predicted the experimental results for three of the five conditions examined. It was theorised that differences in behaviour at the genus level might explain why all five conditions were not successfully predicted.

In an attempt to improve the prediction capability, the ‘black-box’ of mixed genus analysis was ‘opened’ to allow the effects of variables on phosphorus accumulation at the genus level to be directly examined. To achieve this, a new image analysis method was developed that quantified stained polyphosphate granules in individual algal cells. To ensure the granules

being measured were indeed polyphosphate, algal cells were analysed using transmission electron microscopy coupled with energy dispersive X-ray spectroscopy, which confirmed the granules contained higher levels of phosphorus compared to the remaining cell. The image analysis method was then used to quantify stained polyphosphate granules in individual cells from the 40 batch factorial experiments mentioned previously.

The results using the image analysis method showed that, for the five most abundant algal genera, *Micractinium/Microcystis* had the highest average accumulation of polyphosphate granules (17% μm^2 granule/μm^2 cell), followed by *Scenedesmus* (12%), *Pediastrum* (11%), *Monoraphidium* (8%), and *Actinastrum* (4%). Although none of the genera studied had the same combination of significant variables, all five genera preferred a high phosphorus concentration to elevate polyphosphate granule accumulation. Furthermore, a high light intensity, high organic load, or high temperature was preferred by the algae if the variable was significant for that genus.

The culture used in the bench-scale continuous flow 'luxury uptake' process originated from a mixed genus WSP culture; however, it had become dominated by the *Scenedesmus* genus. Therefore, the regression equation was refined to use the batch data for this genus alone. This new *Scenedesmus* regression equation was compared against the experimental data from the 'luxury uptake' process previously mentioned. Polyphosphate granule accumulation was now successfully predicted in all five experimental conditions at the 95% confidence level. This improved prediction capability indicates that an understanding of the algal genus present in a WSP system is required for accurate predictions of the phosphorus accumulation to be obtained, and the batch data can indeed be applied to a continuous process.

An unexpected result of the research was that, contrary to what was believed in the literature, an increase in the phosphorus content in the biomass did not necessarily increase the polyphosphate granule accumulation. Further examination identified that individual cells from the same algal species had varying polyphosphate granule contents from 0% to over 20% (μm^2 granule/μm^2 cell) when exposed to the same conditions. This variation was hypothesised to be from cellular functions influencing the granules differently depending on the individual alga's cell cycle. In addition, when the phosphorus content in the biomass was increased above 2.1% (gP/gSS), no significant effect on the average quantity of polyphosphate granules was observed. This finding indicates that other forms of phosphorus storage must be responsible for attaining a highly elevated phosphorus content in the biomass.

The findings in this thesis have demonstrated that manipulation of phosphorus accumulation in WSP algae is possible, and predictable, albeit at a genus level. These findings pave the way forward for the development of a new algal-based biotechnology capable of harvesting phosphorus from wastewater.

Acknowledgements

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Structure of the thesis

The chapters in this thesis are adapted from a series of scientific papers that have been published or are ready for submission to international peer-reviewed journals. While the presented content of the chapters is the same as the papers they are based on, the following changes have been made to improve the clarity of the thesis:

- Formatting changes have been conducted to ensure a consistent style throughout the thesis,
- The introductions of each chapter have been shortened to reduce repetition,
- If a method is repeated in later chapters, a reference back to the first mention of the method has been used, and
- Changing the references of papers produced in this PhD to their corresponding chapter number (i.e. a reference to the paper Sells *et al.* (2018) has been changed to Chapter 2).

A preface has been included at the beginning of each chapter to help link the individual chapters together and illustrate their contribution towards the research objectives of this thesis. The content presented in Chapters 1 through 5 has been used to produce the thesis conclusions that are discussed after Chapter 5.

The structure of this thesis complies with the Massey University "Guidelines for Doctoral Thesis by Publications", 2015 issued by the graduate research school (GRS).

List of papers and contribution

A list of the chapters and relevant publications are given below. Along with these publications, an overview of the initial findings of this thesis was presented at the "11th IWA Specialist Group Conference on Wastewater Pond Technologies, 2016" in Leeds, UK.

Chapter 2

Sells, M. D., Brown, N., & Shilton, A. N. (2018). Determining variables that influence the phosphorus content of waste stabilization pond algae. *Water Research, 132*, 301-308. doi:10.1016/j.watres.2018.01.013

Chapter 3

Sells, M.D., Brown, N. and Shilton, A.N. Interactions between environmental and process variables influence phosphorus accumulation in waste stabilisation pond algae. *In preparation for submission to Water Research.*

Chapter 4

Sells, M.D., Brown, N. and Shilton, A.N. The conditions for phosphorus accumulation in algae are genus dependent. *In preparation for submission to Ecological Engineering.*

Chapter 5

Sells, M.D., Brown, N. and Shilton, A.N. Relating polyphosphate granule accumulation to the algal phosphorus content. *In preparation for submission to Environmental Science and Technology.*

Matthew Sells was the main contributor and lead author on all the papers mentioned above, with advice and editing assistance being obtained from his supervisors Prof. Andrew Shilton and Dr. Nicola Brown. Matthew Sells designed, conducted, and analysed the experimental work. The "statement of contribution to doctoral thesis containing publications" for the published paper can be found at the end of the appendices.