CRANFIELD UNIVERSITY

Rachel Louise Whitton

Algae Reactors for Wastewater Treatment

School of Energy, Environment and Agrifood

EngD
Academic Year: 2015 - 2016

Supervisors: Prof. Bruce Jefferson & Dr Raffaella Villa
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Abstract

The onset of the Water Framework Directive (WFD) will challenge water utilities to further reduce their wastewater phosphorus discharges to < 0.5 $mg.L^{-1}$. Whilst conventional treatments, such as chemical dosing, are able to meet these new discharge consents, the strategies are representative of a linear economy model where resources are unrecovered and disposed.

An alternative solution which can contribute to the aspiration of a circular economy is microalgae. Microalgae are ubiquitous in wastewater environments and assimilate phosphorus during their growth, to residual concentrations complementary of the WFD. Furthermore, microalgal biomass can be anaerobically digested to produce biomethane offering the potential for an energy neutral approach. However, uptake of microalgal systems are lacking in the UK through limited knowledge of operation; and the belief that such solutions are synonymous to large, shallow open ponds with extensive treatment times. The development of alternative microalgal reactors are increasingly investigated to overcome these implementation challenges. Of these, immobilised microalgae has shown great potential; and whilst within its infancy demonstrates the greatest opportunity for development and optimisation.

This thesis determines the critical operational parameters that influence the remediation efficacy of immobilised microalgae for tertiary nutrient removal; including species selection, biomass concentration, treatment period and lighting; with recommendations for optimal performance. These recommendations are then applied to the design and operation of an immobilised bioreactor (IBR) to understand the key design and operating components that influence the overall economic viability. In doing so, the potential for an IBR to be economically viable, within the next decade, in comparison to traditional approaches are discussed.

Keywords: immobilisation, phosphate, nitrogen, circular economy

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'All you people can't you see, can't you see? How your love's affecting our reality.
Every time we're down, you can make it right, and that makes you larger than life'
Backstreet Boys (1999)

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Abbreviations

AD	Anaerobic Digestion
ADP	Adenosine Diphosphate
AMP	Asset Management Plan
AOB	Ammonia-Oxidising Bacteria
AS	Activated Sludge
ATP	Adenosine Triphosphate
AW	Aerated Wetlands
BMP	Biomethane Potential
BOD	Biological Oxygen Demand
CAPEX	Capital Cost
CCAP	Culture Collection of Algae and Protozoa
CHP	Combined Heat and Power
COD	Chemical Oxygen Demand
CPI	Consumer Price Index
DAF	Dissolved Air Flotation
DI	Deionised Water
DRP	Dissolved Reactive Phosphorus
DW	Dry Weight
EBPR	Enhanced Biological Phosphate Removal
H&S	Health and Safety
HRAP	High Rate Algal Pond
HRT	Hydraulic Retention Time
IBR	Immobilised Microalgal Bioreactor
IDC	Industrial Doctorate Centre
LED	Light Emitting Diodes
LF	Lang Factor
OPEX	Operational Cost
PAR	Photosynthetic Active Radiation
PBR	Photobioreactor
PE	Population Equivalent
PFD	Photon Flux Density
RABR	Rotating Algal Biofilm Reactors
RI	Risk Index
SSSI	Site of Special Scientific Interest

TPBR	Tubular Photobioreactor
TSS	Total Suspended Solids
UASB	Upflow Anaerobic Sludge Blanket
UK	United Kingdom
UWWTD	Urban Wastewater Treatment Directive
VS	Volatile Solids
WFD	Water Framework Directive
WLC	Whole Life Cost
WWTW	Wastewater Treatment Works

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Chapter 1. Introduction

1.1 Background

The onset of the water framework directive (WFD) across Europe will require further reductions in wastewater phosphorus (P) discharges to below the current 1 - 2 $mg.L^{-1}$ specified within the Urban Wastewater Treatment Directive (UWWTD) (European Community, 1991), to 0.5 $mg.L^{-1}$ with some sites expected to be as low as 0.1 $mg.L^{-1}$ (A. Brookes & P. Vale, 2011, pers. comms., 20 October). Polishing wastewater effluent to meet the present UWWTD discharge requirement is most commonly achieved through the addition of a metal coagulant, such as ferric chloride ($FeCl_3$), to precipitate and aggregate residual P into particles or alternatively; biological removal through uptake by polyphosphate accumulating organisms as part of an enhanced biological phosphate removal (EBPR) strategy consisting of anaerobic and aerobic processes.

The aggregates formed are subsequently removed following filtration and/or settlement producing a sludge which can either be utilised as an additional resource or ultimately disposed (Yeoman et al., 1988). How the sludge is used is largely dependent on the sludge characteristics (Jenkins et al., 1971; Zhou et al., 2008) with 37% of the total sludge produced in Europe applied to land as a fertiliser, with the remaining mostly incinerated or landfilled (European Commission, 2009) largely through the association of a metal coagulant rendering the recovered P unavailable.

The impending reductions in wastewater P concentrations can be satisfied through the use of coagulation, however the quantity of coagulant required to further reduce P to below 1$mg.L^{-1}$ is not a linear relationship. A dosage up to three times the current quantity is necessary resulting in a subsequent increase in the production of residual sludge. This poses potential challenges to wastewater treatment works (WWTW) that do not currently incorporate chemical dosing (such as small rural works), requiring improved infrastructure for the transport of chemicals and residual sludge on and off site; inclusion of chemical storage facilities and provision of health and safety (H&S) facilities such as safety showers supplied with potable water (Germain-Cripps, 2015).

Whilst coagulation supports satisfactory remediation of wastewater P and protects the receiving water body of environmental impacts associated with increased nutrient

concentrations i.e. eutrophication, the treatment strategy aligns to current linear economy approaches. Potential resources are unrecovered and ultimately disposed representative of a consumable environment, which may prove to be unsustainable in the long term future. As such, there is a desire to move towards technology options/strategies that not only protect the local environment receiving the treated effluent, but offer wider environmental benefits through resource recovery and/or energy generation and hence switch to an approach aligned to circular economy thinking.

Overall the uncertainties surrounding the future cost of coagulant (Keeley et al., 2014); the understanding that residual metal consents will also tighten (A. Brookes, 2014, pers. comms., 20 October); considerations required in relation to sludge disposal; challenges faced by WWTW to incorporate additional chemical dosing strategies, and the desire to close the resource loop, highlights the need for an alternative solution for P polishing for the forthcoming changes in consent.

1.1.1 Microalgae for wastewater nutrient remediation

Microalgae are photosynthetic organisms found within the aquatic environment, characterised by either a single cell or multicellular/filamentous conformation (Lyon et al., 2015). Positive attributes set them apart from other biological species such as; their relatively fast growth rates, ability to exist at concentrations which can exceed 10^6 cells.mL^{-1} (non-filamentous) and their need to assimilate CO_2 and macronutrients, such as P and nitrogen (N) during their growth (Christenson and Sims, 2011), which can be supplied from wastewater effluents (Chiu et al., 2015). In addition, microalgal biomass can be anaerobically digested following a suitable pre-treatment (Ometto et al., 2014) to produce bio-methane offering the potential for an energy neutral approach to wastewater nutrient removal.

Microalgae are ubiquitous in wastewater environments (Davis et al., 1990) albeit at dilute concentrations, confirming the nutrient characteristics of such environments are suitable for growth (Xin et al., 2010). Promising P remediation characteristics have been demonstrated with up to 99% removal efficiency under optimised conditions with microalgae treating primary effluent within an open pond environment (Nurdogan and Oswald, 1995). Furthermore, microalgae simultaneously remediate N species in preference of NH_4^+ > NO_3^- > Org-N (Lau et al., 1995) thereby contributing to both

nitrification and denitrification and offering the potential for a complete nutrient remediation solution.

1.1.2 Understanding the route to implementation

Whilst algae to provide an attractive alternative solution for nutrient removal the exact path to implementation is less clear. Various reactor designs are available and include either suspended or non-suspended systems with sub-categories of either open or closed to the environment; with example reactors including (but not limited to) high rate algal ponds (HRAP); photobioreactors (PBR); attachment systems including floways and substrate submersion; and matrix-immobilisation (further design and performance details can be found within Chapter 2).

With the majority of WWTW within the UK categorised as small (treating a population equivalence (PE) of < 2,000, (Upton et al., 1995)), certain attributes are desirable in regards to the design and performance of a microalgal bioreactor before considering retrofitting to an existing WWTW with the intention of polishing additional P to meet the future WFD. These attributes include; (1) low technology footprint due to potentially limited land availability around existing WWTW; (2) treatment time (< 1 day) to coincide with upstream processes enabling constant output and flow as previously attained by the works; (3) a residual P concentration of < 0.5 $mg.L^{-1}$ to satisfy the forthcoming requirements of the WFD; and (4) economically comparable to a conventional treatment process upon integration in to a process flow sheet considering any benefits (e.g. N remediation, biogas production) which may be gained and contribute to a circular economy.

An assessment of the available microalgal bioreactors was undertaken to evaluate their ability to meet the desired attributes (Table 1.1) in conjunction with a review of the literature (Chapter 2) to enable the selection of a suitable and practical technology for implementation by the water utilities.

Whilst the technology is within its infancy, matrix-immobilisation was selected as the focus of this research. The technology has the greatest opportunity for development, whilst performing well within initial laboratory trials and satisfying the required attributes. Furthermore the use of a granular like media i.e. the immobilised beads, was

found to be similar to known treatment processes (e.g. packed and fluidised beds) which are used within water utilities with a long history of experience and knowledge thereby reducing potential perception barriers. As such, research was undertaken on the application of an immobilised microalgal bioreactor (IBR) to provide a sound basis for adoption and ensure any economic and environmental benefits can be realised.

Table 1.1 Desired attributes for the implementation of a microalgal bioreactor for wastewater nutrient remediation.

Bioreactor solution	Desired attributes for implementation							
	Low footprint		Treatment time < 1 day		<0.5 mg.L^{-1} P residual		Key economic challenges	
HRAP	✘	Large footprint (1.25 ha.pond^{-1}) through low photosynthetic efficiency and associated reduced biomass concentration (Gupta et al., 2015).	✘	4 – 10 days (Craggs et al., 2012; Sutherland et al., 2014).	✓	< 0.5 mg.L^{-1} (Picot et al., 1992).	✘	Inexpensive to install and operate but with intensive and expensive techniques required for harvesting suspended biomass.
PBR	✘ ✓	Medium sized footprint through reduced biomass concentration and low photosynthetic efficiency.	✘ ✓	1 – 7 days (Cromar and Fallowfield, 1997).	✓	< 0.5 mg.L^{-1} (Di Termini et al., 2011).	✘	Expensive to install and operate (Ruiz et al., 2013) with intensive and expensive techniques required for harvesting suspended biomass.
Attached	✘	Large footprint (1012 m^2) through low photosynthetic efficiency (Adey et al., 1993; Craggs, 2001).	✘	> 6 days (Johnson and Wen, 2010).	✓	0.5 mg.L^{-1} (Guzzon et al., 2008).	✓	Inexpensive to install and operate with high effort and manually intensive yet inexpensive biomass harvesting through physical detachment e.g. scrapping the attachment surface (Gross et al., 2015).
Matrix-immobilisation	✓	Low footprint through intensified biomass concentration (Chevalier and De la Noue, 1985).	✓	6 – 12 h (Filippino et al., 2015).	✓	< 0.5 mg.L^{-1} (Filippino et al., 2015).	?	Capital and operational costs unknown, inexpensive biomass harvesting through gravity settlement.

1.2 Research Development

The work presented in this thesis was developed as part of the STREAM Industrial Doctorate Centre (IDC) in conjunction with the sponsoring partners Anglian Water, Severn Trent Water and Scottish Water.

The use of microalgae for nutrient remediation has been gaining considerable attention, with the industrial partners approached by numerous research groups promoting collaboration for funding and on-site trials. However, there was uncertainty as to whether microalgae would be a viable solution for the partners and the UK water industry as a whole, and if investment would be beneficial. As such, the research was developed to investigate the viability of microalgae as an alternative polishing system in the remediation of P and NH_4 from tertiary wastewater effluent. Following favourable results throughout the course of the research, the brief evolved from an evaluation of viability to the development and optimisation of a microalgal reactor which would satisfy design and performance requirements, and represent a potentially economically viable solution in comparison to conventional solutions for nutrient polishing.

Following an evaluation of microalgal solutions, matrix-immobilisation was selected for further research and development. Focus was placed on the key design and operating variables that would influence overall economic viability, linked to a mechanistic understanding of nutrient remediation with algae to provide a sound basis for defining a business case for implementation of the technology.

1.3 Aims and Objectives

The overall aim of the research was to understand and critically evaluate the technical and economic challenges associated with implementation of technologies utilising freshwater microalgae as a nutrient polishing process within wastewater treatment.

As such it was hypothesised that through the optimisation of key design variables, an IBR has the potential to remediate tertiary wastewater nutrients to the required residual concentrations whilst remaining economically viable. As such microalgae could be considered an alternative solution to nutrient remediation and a candidate solution in the advancement of a closed resource loop within wastewater treatment. To test the hypothesis and deliver against the overall aim the following objectives were set:

Objective 1. To produce a state of the art critical review on microalgal technologies for nutrient remediation to inform the selection of a technology for further research and development.

Objective 2. To determine whether microalgal character can be linked to nutrient removal abilities within low nutrient concentration environments (consistent with tertiary treatment) and inform species selection.

Objective 3. To determine the critical operational parameters that influence the performance efficacy of IBR technology for tertiary nutrient removal.

Objective 4. To understand they key design and operating components that influence the overall economic viability of the technology and in doing so understand the potential for an IBR to be economically viable in comparison to traditional approaches.

A conceptual diagram of the integration of an IBR for tertiary treatment at a WWTW is presented with the objectives highlighted (Figure 1.1).

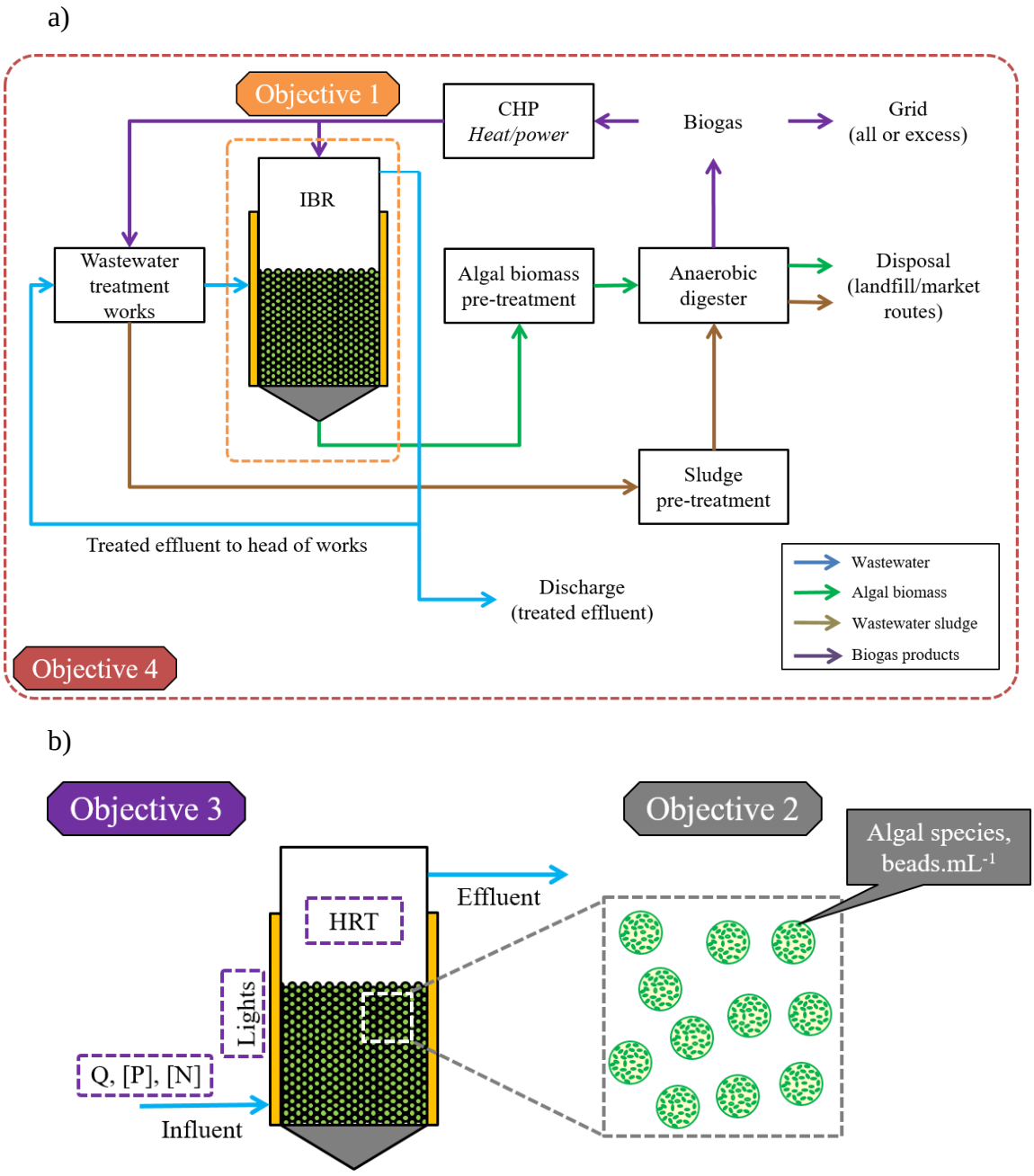

Figure 1.1 a) The integration of an IBR into a flowsheet for tertiary wastewater treatment; and b) an IBR unit, highlighting thesis objectives.

1.4 Thesis Plan

This thesis is presented as a series of chapters formatted as journal papers. All papers were written by the primary author, Rachel L. Whitton and edited by Prof. Bruce Jefferson and Dr Raffaella Villa. All experimental work was designed, co-ordinated and completed by Rachel L. Whitton at Cranfield University (UK) with contributions from PhD, MSc and visiting students as follows. Microalgal cultivation and harvesting was undertaken in collaboration with Dr Francesco Ometto, formerly of Cranfield University (UK). Characterisation of microalgal species in relation to nutrient remediation potential and internal composition described in Chapter 3 was assisted by Amandine Le Mével of Ecole Nationale Superieure de Chimie de Rennes (FR), with support provided in the culturing of all chosen species and sample analysis throughout the trials. In addition, assistance in sample collection and analysis during the continuous trials for five of the twelve experimental regimes described in Chapter 4 were aided by Martina Santinelli of Marche Polytechnic University (IT).

Initially, a literature review was completed on the current microalgal technologies available for wastewater nutrient remediation. Remediation performance linked to microalgal removal mechanisms in addition to design and operational parameters of bioreactors were assessed and discussed. The review provides increased confidence in the use of microalgae for the remediation of wastewater effluents to < 0.5 $mgP.L^{-1}$ and highlights the potential of non-suspended systems. As such a non-suspended system; matrix-immobilisation, was carried forward for further research and development (Chapter 2, Paper 1 – published: Whitton, R., Ometto, F., Pidou, M., Jarvis, P., Villa, R. and Jefferson, B. Microalgae for Municipal Wastewater Nutrient Remediation: Mechanisms, Reactors and Outlook for Tertiary Treatment (2015), *Environmental Technology Reviews*, vol. 4, no. 1, pp. 133-148).

The outcome of Chapter 2 informed the selection of an appropriate microalgal technology, but not the selection of a microalgal species. Chapter 3 characterises and relates a freshwater microalgal species nutrient remediation potential to the species internal phosphorus and nitrogen composition. This relationship enables the selection of a species depending on the characteristics of the wastewater to be treated. Findings gave an insight into species selection in addition to biomass concentrations required to achieve

the necessary levels of remediation. The findings for microalgal selection and biomass requirements were then translated to the immobilisation technology (Chapter 3, Paper 2 – published: Whitton, R., Le Mével, A., Pidou, M., Ometto, F., Villa, R. and Jefferson, B. Influence of Microalgal N and P Composition on Wastewater Nutrient Remediation, *Water Research*, vol. 91, pp. 371-378).

Chapter 4 then optimises the immobilisation technology, through the critical operating parameter of hydraulic retention time (HRT) for the remediation of wastewaters of varying N:P ratios under continuous treatment (Chapter 4, Paper 3 – in preparation: Whitton, R., Santinelli, M., Pidou, M., Ometto, F., Henderson, R., Roddick, F., Jarvis, P., Villa, R. and Jefferson, B. Tertiary Nutrient Removal from Wastewater by Immobilised Microalgae: Impact of N:P Ratio and Hydraulic Retention Time (HRT), *Chemical Engineering Journal*). Findings further support an IBR for the treatment of P to residual concentrations of <0.5 $mg.L^{-1}$ for varying wastewaters achieved within HRTs of 3 – 20 h, representing a reduced treatment period (and associated reactor footprint) in comparison to alternative microalgal solutions.

Further, the importance of lighting design parameters was critically evaluated (Chapter 5, Paper 4 – in preparation: Whitton, R., Pidou, M., Ometto, F., Jarvis, P., Villa, R. and Jefferson, B. The Effect of Light on Wastewater Nutrient Remediation by Immobilised Microalgae, *Water Research*), with performance analysed under lighting regimes of differing wavelengths, intensities and photoperiods to enable recommendation of a suitable light regime and implications when incorporated into an IBR reactor design.

Findings from the previous chapters were then incorporated to the design of an IBR for tertiary P remediation for a 2,000 PE and compared against conventional solutions for P polishing in addition to alternative microalgal technologies (Chapter 6, Paper 5 – in preparation: Whitton, R., Pidou, M., Ometto, F., Villa, R. and Jefferson, B. Understanding the Implementation Challenges of using an Immobilised Microalgal Bioreactor for the Remediation of Wastewater Nutrients: An Economic Assessment, *Algal Research*). Cost critical components and processes in the implementation of an IBR were determined and historical cost data analysed to establish the impact of predicted changes in the price of the key cost components, enabling the eventual cost effective implementation of an IBR in comparison to the alternative technologies examined.

Chapter 7 is an overall discussion of the implications of the work and the suitability of microalgal reactors for wastewater nutrient removal in the UK. The benefits of an IBR over alternative microalgal solutions, and suitability in comparison to alternative P solutions are discussed, whilst highlighting the key implementation challenges which still remain.

Finally, Chapter 8 summarises the key conclusions and recommends additional areas of work to further develop an IBR for wastewater nutrient remediation. Table 1.2 summarises this thesis plan and the status of paper submissions.

Table 1.2 Thesis plan and status of paper submissions.

Chapter	Paper	Objective	Title	Journal	Status
2	1	1,2,3	Microalgae for Municipal Wastewater Nutrient Remediation: Mechanisms, Reactors and Outlook for Tertiary Treatment	Environmental Technology Reviews	*Environmental Technology Reviews*, 4 (1), 133-148
3	2	2	Influence of Microalgal N and P Composition on Wastewater Nutrient Remediation	Water Research	*Water Research*, 91, 371-378.
4	3	3	Tertiary Nutrient Removal from Wastewater by Immobilised Microalgae: Impact of N:P Ratio and Hydraulic Retention Time (HRT)	Chemical Engineering Journal	In preparation
5	4	3	The Effect of Light on Wastewater Nutrient Remediation by Immobilised Microalgae	Water Research	In preparation
6	5	4	Understanding the Implementation Challenges of using Immobilised Microalgal Bioreactor for the Remediation of Wastewater Nutrients: An Economic Assessment	Algal Research	In preparation
7	--	1, 2, 3, 4	Implications of the Work: Overall Perspective on the Appropriateness of Algae Reactors for Wastewater Treatment in the UK	--	--
8	--	--	Conclusions and Future Work	--	--

1.5 References

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Chapter 2. Microalgae for Municipal Wastewater Nutrient Remediation: Mechanisms, Reactors and Outlook for Tertiary Treatment

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Abstract

This review explores the use of microalgae for nutrient removal in municipal wastewater treatment considering recent improvements in the understanding of removal mechanisms and developments of both suspended and non-suspended systems. Nutrient removal is associated to both direct and indirect uptake with the former associated to the biomass concentration and growth environment (reactor). Importantly, direct uptake is influenced by the nitrogen:phosphorus (N:P) content in both the cells and the surrounding wastewater with opposite trends observed for N and P. Comparison of suspended and non-suspended systems revealed that whilst all where capable of achieving high levels of nutrient removal, only non-suspended immobilised systems could do so within reduced hydraulic retention times of less than 1 day. As microalgae are photosynthetic organisms, the metabolic processes associated with nutrient assimilation are driven by light. Optimisation of light delivery remains a key area of development with examples of improved mixing in suspended systems and the use of pulsating lights to enhance light utilisation and reduce costs. Recent data provides increased confidence in the use of microalgae for nutrient removal in municipal wastewater treatment enabling effluent discharges below 1 $mg.L^{-1}$ to be met whilst generating added value in terms of bio products for energy production or nutrient recovery. Ultimately, this review suggests future research will focus on non-suspended systems and the determination of the added value potential. In so doing it is predicted that microalgae systems will be significant in the delivery of the circular economy.

Keywords: nitrogen, phosphorus, bioreactor, suspended, non-suspended

2.1 Introduction

The remediation of inorganic nitrogen and phosphorus from wastewater by microalgae is well documented e.g. Oswald and Gotaas, 1957; Bogan et al., 1960; Gates and Borchardt, 1964; Doran and Boyle, 1979; Hashimoto and Furukawa, 1989; Davis et al., 1990a; Jiménez-Pérez et al., 2004; Boelee et al., 2011; Abinandan and Shanthakumar, 2015, and considered an environmental approach to nutrient polishing (Shi et al., 2007; Christenson and Sims, 2011). In addition to enabling low nutrient discharges a number of added benefits have been described; a) sequestering of CO_2 from the atmosphere during photosynthesis (Oswald and Golueke, 1960); b) oxygenating the treated effluent (Silva et al., 2015); c) unlike alternative biological treatment processes, a compulsory inorganic carbon source is unnecessary to optimise treatment (Boelee et al., 2011; Silva et al., 2015) and d) removal of trace organic micropollutants. Furthermore, following treatment the algal biomass can be processed for the production of low value products, within human and animal nutrition, cosmetics and biofuels, including biomethane through anaerobic digestion of residual biomass (Spolaore et al., 2006).

Microalgae are ubiquitous to wastewater environments (Davis et al., 1990b) albeit at dilute concentrations, confirming the nutrient characteristics of such environments are suitable for growth (Xin et al., 2010) with microalgae demonstrating the ability to remediate effluents at concentrations commonly encountered post secondary treatment (Judd et al., 2015). Microalgae are therefore considered as prospective candidates for tertiary wastewater treatment (Sukačová et al., 2015; Gómez-Serrano et al., 2015; Selvaratnam et al., 2015).

The desired features for a microalgal solution for tertiary treatment includes performance reaching the required level of remediation within a practical hydraulic retention time (HRT). The longest HRTs typically encountered in tertiary treatment are related to constructed wetlands extending up to 1 day (Butterworth et al., 2013). Current operation of microalgae treatment is most commonly achieved in high rate algal ponds (HRAP) with HRTs of 4 - 10 days. Accordingly, uptake is predominately reported in locations where land availability is not restrictive (e.g. USA (Cai et al., 2013)) but represents a research challenge to broaden uptake.

The combination of microalgae being able to meet low nutrient discharges (e.g. sub 1.0 $mg.L^{-1}$ total phosphorus) and the generation of added value components (e.g. biomethane feedstock material) have resulted in refreshed consideration of the need and benefits of microalgae over traditional nutrient removal options (e.g. chemical dosing for phosphorus), which offer no added value. Illustration of this is seen in regard to phosphorus, where a range of new technologies being implemented to meet sub 1.0 $mg.L^{-1}$ discharges are all based on chemical dosing and clarification (e.g. BluePro, CoMag), resulting in an increase in coagulant use and residual sludge production. Additional issues arise at sites that previously did not incorporate chemical dosing such as small rural works. In such cases, the chemical dosing based options generate additional challenges due to the need for better infrastructure around transport (roads) and health and safety, including the supply of potable water for safety showers and chemical storage facilities (Germain-Cripps, 2015). Accordingly, there is a need for microalgae treatment options that offer a real alternative to the chemical dosing based technologies beyond sites where use of HRAP is appropriate (Jefferson, 2015; Vale, 2015). This has seen a growth in research around application to more nutrient limited environments such as tertiary treatment (Sukačová et al., 2015) coupled with new insights and trials of alternative technologies (Gupta et al., 2015; Zeng et al., 2015). This review aims to appraise the new insights and technologies to consider the impact on the future potential for microalgae systems for tertiary treatment.

2.1.1 Overview of Microalgal Nutrient Remediation Mechanisms

2.1.1.1 Direct (Biological) Remediation of Nitrogen and Phosphate and Microalgal Species' N:P Composition

Nutrient remediation with microalgae occurs through one of two pathways (Figure 2.1). Direct remediation is the most commonly discussed mechanism of remediation and is achieved through interconnected biochemical pathways for the uptake of the target nutrients into the biomass for storage (Powell et al., 2008; Ruiz-Martínez et al., 2015), or assimilation into nucleic acids and proteins for biomass growth (Cai et al., 2013) (Figure 2.1).

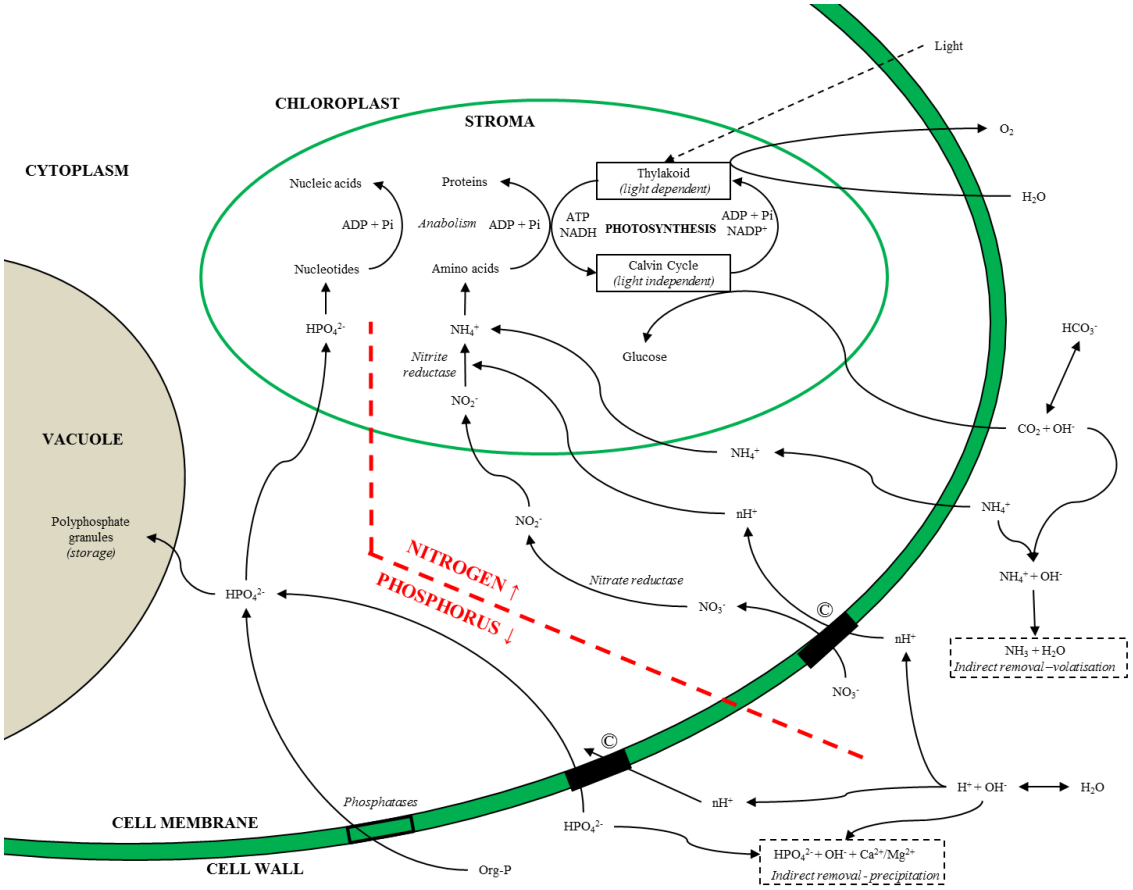

Figure 2.1 Schematic diagram of a microalgal cell summarising the biochemical pathways of nitrogen and phosphorus remediation, including indirect mechanisms (highlighted within a dashed box). © represents co-transportation.

Inorganic nitrogen i.e. nitrite (NO_2^-), nitrate (NO_3^-) and ammonium (NH_4^+) are translocated across the cell membrane (Cai et al., 2013) in the preference of $NH_4^+ > NO_3^-$ > Org-N (Lau et al., 1995). These oxidised nitrogen species are subsequently reduced to NH_4^+ and assimilated into amino acids for the formation of proteins (Figure 2.1) with NH_4^+ uptake preferred due to the reduced energy requirement necessary for reduction and assimilation (Cai et al., 2013). Accordingly, microalgae can be utilised for total nitrogen removal (nitrification and denitrification), with NO_3^- assimilation observed following the uptake of NH_4^+ (Maestrini et al., 1986) from the source wastewater.

Phosphate, in the preferred form of $H_2PO_4^-$ and HPO_4^{2-}, is transported across the cell membrane via energised transport (Cai et al., 2013; Silva et al., 2015) and assimilated into nucleotides following phosphorylation for the synthesis of ribosomal RNA (Beuckels et al., 2015) (Figure 2.1). A nitrogen source is therefore required for the synthesis of

proteins to enable the assimilation of phosphorus, with a limitation of either nutrient resulting in a low cell protein content and reduced biomass growth (Beuckels et al., 2015). Furthermore, in high phosphate environments, microalgae can consume excess phosphate through a luxury uptake pathway (Eixler et al., 2006; Powell et al., 2008) for storage as an acid-insoluble polyphosphate granule (Powell et al., 2008) (Figure 2.1) for future use in times when the external phosphate concentration may become limiting.

Nutrient uptake by microalgae depends on the associated concentration in the microalgae biomass such that phosphorus removal is consistently lower than nitrogen (Table 2.1) due to a greater microalgal nitrogen biomass content (Nurdogan and Oswald, 1995). Freshwater microalgae biomass N:P molar concentrations range between 8:1 – 45:1 indicating the importance of species selection when optimising treatment (Hecky et al., 1993). In addition, microalgae are known to adjust the N and P concentration in their biomass in relation to the levels in the surrounding medium (Beuckels et al., 2015; Choi and Lee, 2015). For example, when cultured in mediums of varying N:P, an internal N:P content of 8.5 – 32 and 4.1 – 32 have been reported for the freshwater species *Chlorella vulgaris* and *Scenedesmus obliquus* (Rhee, 1974; Oh-Hama and Miyachi, 1988; Beuckels et al., 2015).

The remediation of nitrogen and phosphate are generally correlated to both an increase in biomass volume (Xin et al., 2010) and the internal nutrient content within the biomass (Portielje and Lijklema, 1994; Beuckels et al., 2015). Biomass productivity (growth) decreases with an increasing external N:P concentration as illustrated in the case of *C.vulgaris* with a maximum growth rate of 2.97 $g.L^{-1}.d^{-1}$ at N:P ($mg.mg^{-1}$) >10 demonstrated through a transition from N to P limitation (Choi and Lee, 2015). In relation to phosphorus, an inverse relationship between a species internal content and specific uptake rate is reported (Ruiz-Martínez et al., 2015; Choi and Lee, 2015). This results in reduced nutrient removal as illustrated with a culture of *C.vulgaris*, where phosphorus remediation decreased from >80% with an increasing P cellular content when treating wastewater with an N:P up to 20 $mg.mg^{-1}$ down to only 20% when treating a wastewater with a nutrient ratio of >50 N:P (Choi and Lee, 2015). Similarly, Ruiz-Martínez, (2015) found an increased phosphate uptake rate of ~3.3 $mgPO_4\text{-}P.gTSS^{-1}.h^{-1}$ in comparison to ~0.7 $mgPO_4\text{-}P.gTSS^{-1}.h^{-1}$ for *Scenedesmus* sp. with internal P concentrations of ~0.6 and

1% (w/w) respectively. An internal phosphate concentration of <1% characterises growth within P limited environments (Hessen et al., 2002) , whereas >1% is indicative of microalga luxuriously consuming phosphate for growth and storage for future use in conditions of limitation (Powell et al., 2008). Observations from these studies suggest an increased specific uptake rate for phosphorus is a result of either a limiting external concentration or an initially reduced internal content, with both conditions representing times of stress when P assimilation is necessary for the continued metabolic processes and growth of a culture. The opposite has been demonstrated for nitrogen assimilation, with a cellular content of ~0.02 $mgN.mgVSS^{-1}$ for *C.vulgaris* when remediating an influent profiled by an N:P ($mg.mg^{-1}$) of 30 in comparison to 0.2 $mgN.mgVSS^{-1}$ at an N:P of 80 (Choi and Lee, 2015) corresponding with P limitation. Similarly, the nitrogen removal efficiency of *Scenedesmus* sp. has been reported to decrease significantly when the N:P ratio exceeds 15:1 (Li et al, 2010). Whilst no significant link has been observed between P removal and carbon concentrations, below a C:N of 10, nitrate uptake has been observed to be reduced in the case of *Chlorella* sp. (Wu et al, 2015).

The ability of a range of microalgae to remove nutrients from wastewater and synthetic wastewater has been analysed extensively within laboratory trials. The majority of research to date has been conducted on unicellular chlorophyceae (Chevalier and De la Noue, 1985), in particular from the *Chlorella* and *Scenedesmus* families (Table 2.1). Species from these families are largely used due to their dominance in freshwater environments (Tang et al., 1997), the ease in which they are cultured and reproduce (Kim et al., 2010; Gómez-Serrano et al., 2015) and their ability to efficiently remove nutrients (Tang et al., 1997). Recent comparisons of a range of algae have largely confirmed the suitability of *Scenedesmus* sp. for use in tertiary treatment applications (Gómez-Serrano et al., 2015).

However, unicellular algae are difficult to harvest resulting in recent research into non planktonic algae, especially filamentous species such as *Oedogonium* sp and *Tribonema* sp (Roberts et al., 2013; Wang et al., 2013). For instance, Liu and Vyverman, (2015) reported that for the filamentous algae trialled, *Cladophora* sp. was most efficient under low N:P ratio wastewater whilst *Pseudanabaena* sp. was better as removing nitrogen from high N:P ratio wastewater. In comparison to research on these identified species, fewer

studies exist on other chlorophyceae as well as other taxa such as cyanobacteria and diatoms. The potential for nutrient removal by other chlorophyceae species in addition to diatoms and cyanobacteria are not as widely investigated, but are known to populate wastewater treatment works (Schumacher et al., 2003; Congestri et al., 2006) and demonstrate beneficial characteristics. Cyanobacteria, for example, contain accessory pigments and an enhanced concentration of chlorophyll in comparison to chlorophyceae, enabling a more efficient use of available light (Cromar and Fallowfield, 1997). In addition, species isolated from colder climates e.g. *Phormidium bohneri*, have shown acceptable growth and nutrient removal rates at cooler temperatures demonstrating removal rates between 2.4 – 19.9 $mg.L^{-1}.d^{-1}$ for NH_4^+ and 1.6 – 13.8 $mg.L^{-1}.d^{-1}$ for TP within secondary effluent (Laliberté et al., 1997). In comparison, optimum nitrogen removal of 77.5 $mg.L^{-1}.d^{-1}$ was observed for *Scenedesmus obliquus* at 31^{o}C with no treatment predicted below 8.8^{o}C based on fitting the observed data to the cordinal temperature model with inflexion (CTMIA) (Ruiz- Martinez et al., 2015).

Mono-culture growth of a species is achieved commercially by operating at favourable loading rates, retention times and environmental parameters (Cromar and Fallowfield, 1997); (e.g. for the growth of *Chlorella*, *Spirulina* and *Dunaliella* (Borowitzka, 1999)); or by the selective recycling of species (Park and Craggs, 2010). However, species control within a wastewater environment is challenging as microalgae are opportunistic (Adey et al., 1993) and attempts to control the community have failed due to contamination from native algal species (Park et al., 2011).

Table 2.1 Ammonium and phosphorus removal by microalgal cultures for varying waste streams.

Algae; Concentration	Design parameters: HRT (d); Flow velocity (?); Scale (m^3)	Design parameters: Aeration	Test conditions: Test waters; Temp (ºC); pH	Test conditions: Irradiance ($\mu mol.m^{-2}.s^{-1}$) [a]; Day length (h)	Influent conc. ($mg.L^{-1}$): NH_4^+	Influent conc. ($mg.L^{-1}$): TP	Removal efficiency (%); Uptake rate: NH_4^+	Removal efficiency (%); Uptake rate: TP	Specific growth rate (d^{-1}); Effluent pH	References
CHLOROPHYCEAE										
Botryococcus braunii --	9 Batch 0.003	50 $mL.min^{-1}$ CO_2	Secondary effluent -- 7.6	-- --	0.17	0.04	99.9[b] --	75.0 --	--	(Sawayama et al., 1992)
Botryococcus braunii --	7 Batch 0.003	50 $mL.min^{-1}$ CO_2	Secondary effluent -- 7.7	-- --	<0.1	0.39	99.8[b] --	97.4 --	--	(Sawayama et al., 1992)
Chlorella vulgaris 253 $mg.L^{-1}$	9 Batch --	--	Wastewater effluent -- 7	100.8 16	7.7	0.9	55.8 --	--	-- ~10	(Kim et al., 2010)
Chlorella vulgaris --	2 Batch 0.0025	Air bubbling	Wastewater effluent 25 --	135 --	--	--	74.3 0.134 $\mu g.h^{-1}.10^{-6}$cells	70.2[d] 0.134 $\mu g.h^{-1}.10^{-6}$cells	0.186 9.0-9.5	(Ruiz-Marin et al., 2010)
Chlorella vulgaris $2x10^6$ $cells.mL^{-1}$	9 Batch 0.002	Air bubbling	Agro-industrial wastewater 20 --	60 --	--	--	--	55.0[d] --	--	(González et al., 1997)
Chlorella vulgaris $5x10^5$ $cells.mL^{-1}$	10 Batch --	0.5 v.v.m filtered air	Primary settled sewage 24 7.1	58[a] 16	35.5	3.9	74.1 --	63.8 --	0.274 --	(Lau et al., 1995)

Algae; Concentration	Design parameters: HRT (d); Flow velocity (?); Scale (m^3)	Design parameters: Aeration	Test conditions: Test waters; Temp (ºC); pH	Test conditions: Irradiance ($\mu mol.m^{-2}s^{-1}$) [a]; Day length (h)	Influent conc. ($mg.L^{-1}$): NH_4^+	Influent conc. ($mg.L^{-1}$): TP	Removal efficiency (%); Uptake rate: NH_4^+	Removal efficiency (%); Uptake rate: TP	Specific growth rate (d^{-1}); Effluent pH	References
Chlorella vulgaris $1x10^6$ cells.mL^{-1}	10 Batch --	0.5 v.v.m filtered air	Primary settled sewage 24 7.1	58[a] 16	35.5.	3.9	97.8 --	87.0 --	0.277 --	(Lau et al., 1995)
Chlorella vulgaris $5x10^6$ cells.mL^{-1}	10 Batch --	0.5 v.v.m filtered air	Primary settled sewage 24 7.1	58[a] 16	35.5	3.9	89.7 --	66.1 --	--	(Lau et al., 1995)
Chlorella vulgaris $1x10^7$ cells.mL^{-1}	10 Batch --	0.5 v.v.m filtered air	Primary settled sewage 24 7.1	58[a] 16	35.5	3.9	99.9 --	78.7 --	--	(Lau et al., 1995)
Scenedesmus dimorphis $2x10^6$ cells.mL^{-1}	9 Batch 0.002	Air bubbling	Agro-industrial wastewater 20 --	60 24	--	--	-- --	55.0[d] --	--	(González et al., 1997)
Scenedesmus obliquus 14 $mg.L^{-1}$ (dm)	7.9 Batch 0.001	160 $mL.min^{-1}$	Wastewater effluent 20 9.3	152[a] 24	27.4	11.8[a]	94 --	98.0 --	0.686 --	(Martínez et al., 2000)
Scenedesmus obliquus 14 $mg.L^{-1}$ (dm)	7.9 Batch 0.001	160 mL.min-1	Wastewater effluent 25 9.3	152[a] 24	27.4	11.8[a]	99 --	98.0 --	0.768 --	(Martínez et al., 2000)

Algae; Concentration	Design parameters: HRT (d); Flow velocity (?); Scale (m^3)	Aeration	Test conditions: Test waters; Temp (ºC); pH	Irradiance ($\mu mol.m^{-2}.s^{-1}$) [a]; Day length (h)	Influent conc. ($mg.L^{-1}$): NH_4^+	Influent conc. ($mg.L^{-1}$): TP	Removal efficiency (%); Uptake rate: NH_4^+	Removal efficiency (%); Uptake rate: TP	Specific growth rate (d^{-1}); Effluent pH	References
Scenedesmus obliquus 14 $mg.L^{-1}$ (dm)	7.9 Batch 0.001	160 $mL.min^{-1}$	Wastewater effluent 30 9.3	152[a] 24	27.4	11.8[a]	99 --	94.0 --	1.051 --	(Martínez et al., 2000)
Scenedesmus obliquus 14 mg.L-1 (dm)	7.9 Batch 0.001	160 mL.min-1	Wastewater effluent 35 9.3	152[a] 24	27.4	11.8a	79 --	54.0 --	0.458 --	(Martínez et al., 2000)
Scenedesmus obliquus --	2.1 Batch 0.0025	Air bubbling	Wastewater effluent 25 --	135 --	32.5	2.5[a]	100[c] 0.180 $\mu g.h^{-1}.10^{-6}$cells	60.0 0.036 $\mu g.h^{-1}.10^{-6}$cells	0.285 9.0-9.5	(Ruiz-Marin et al., 2010)
CYANOBACTERIA										
Phormidium bohneri 100 $mg.L^{-1}$ (dm)	5 Batch 0.02	0.1 v.v.m	Secondary effluent -- --	-- --	--	--	-- 2.4-19.9 $mg.L^{-1}.d^{-1}$	-- 1.6-13.8 $mg.L^{-1}.d^{-1}$	0.190-0.490 8.5-11.1	(Laliberté et al., 1997)

[a] Irradiance units converted to $\mu mol.m^{-2}.s^{-1}$ using conversion guidelines within (Thimijan and Heins, 1983)
[b] Total nitrogen
[c] Nitrate
[d] Orthophosphate
(dm) dry mass.

2.1.1.2 Indirect Nitrogen (Volitisation) and Phosphate (Precipitation) Remediation

A by-product of direct remediation and growth is the alkalisation of the localised environment through; 1) production of hydroxyl radicals (OH^-) during photosynthetic consumption of inorganic carbon such i.e. bicarbonate (HCO_3^-) (Nurdogan and Oswald, 1995; Larsdotter et al., 2007; Kim et al., 2010) and 2) a net uptake of protons (H^+) from the dissociation of H_2O for co-transportation of NO_3^- and PO_4^- through the microalgal cell membrane (Ullrich, 1983; Larsdotter et al., 2007) (Figure 2.1). The modification of the physiochemical environment through pH (Nurdogan and Oswald, 1995) facilitates the method of indirect removal. In the case of NH_4^+, at pH values greater than 7 there is an equilibrium shift within the kinetic equilibria for NH_4^+ and ammonia (NH_3) towards the production of NH_3 (gas) (Martínez et al., 2000) which is subsequently volatised and stripped from the solution. The mechanism of indirect removal of ammonium has been shown to contribute greatly to total NH_4-N remediation with removal percentages of 38 – 100% reported for the cyanobacteria *Phormidium bohneri* (Talbot and de la Noüe, 1993) and 53% - 82% for *Scenedesmus obliquus* under varying temperatures and mixing regimes (Martínez et al., 2000).

Unlike ammonium, phosphate cannot exist in a gaseous state and precipitates with metal ions within the effluent e.g. Ca, Mg and Fe, at elevated pH and high dissolved oxygen concentrations (Powell et al., 2009; Cai et al., 2013) with a removal efficiency of 16 – 63% for *Monoraphidium* species treating sterile-filtered wastewater (Larsdotter et al., 2007). The indirect removal mechanisms are not specifically monitored in the operation of microalgal bioreactors in the vast majority of cases. However, increases from an influent pH ranging between 7 – 9.3 to a final effluent pH between 8.5 – 11.1 are documented during the operation of those bioreactors were the pH is uncontrolled (Supplementary information, Appendix A). Once pH increases beyond 10.5, phosphate precipitation decreases due to a switch towards calcium carbonate formation as a result of the relative change in precipitation kinetics between calcium and phosphate or carbonate (Montastruc et al., 2003).

2.2 Microalgal Bioreactor Configurations for Wastewater Nutrient Remediation

The assimilation of nutrients for growth is facilitated by the process of photosynthesis which is driven by the supply of inorganic carbon, light and temperature (Figure 2.1). Inorganic carbon however, is often regarded as non-limiting (Talbot and de la Noüe, 1993; Cromar and Fallowfield, 1997) within wastewater effluent and expressed indirectly as COD (chemical oxygen demand) (Cromar and Fallowfield, 1997). It is the external factors of light and temperature, as opposed to the concentration of target nutrients, which have the greatest influence on growth and productivity (Talbot and de la Noüe, 1993) and are considered the key design features in the operation of a microalgal bioreactor with studies focusing on light (e.g. Meseck et al., 2005; Kim et al., 2013; Lee et al., 2015); temperature (e.g. Talbot and de la Noüe, 1993; Martinez et al., 1999) in addition to species selection (e.g. Gómez-Serrano et al., 2015; Liu and Vyverman, 2015; Mennaa et al., 2015) to optimise performance.

Varying bioreactor designs are available to enhance growth and facilitate biomass removal following treatment and include suspended and non-suspended systems (Larsdotter, 2006; Christenson and Sims, 2011) with sub-categories of either open to the environment or enclosed (Figure 2.2).

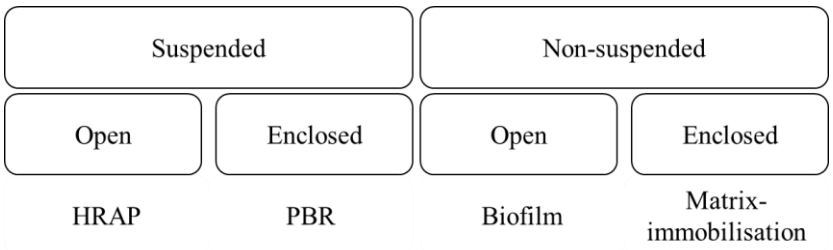

Figure 2.2 Categories of microalgal bioreactors for wastewater remediation.

Suspended cultures enable the microscopic algal cells to move freely within a body of water in dilute concentrations (Zeng et al., 2015) and are most commonly used in microalgal wastewater treatment (Christenson and Sims, 2011). The biomass concentration of suspended systems are reasonably low (<2 $g.L^{-1}$) (Table 2.2) but can exceed 4.5 $g.L^{-1}$ in intensified systems when treating industrial sources (Van Wagenen et al., 2015). If not efficiently removed (harvested) post treatment have been reported to

contribute to an increase in suspended solids content and 60 – 90% of the effluent biological oxygen demand (BOD) (Laliberte et al., 1994).

Harvesting is challenging and expensive and described as the defining factor of overall affordability (Zeng et al., 2015) representing 20 – 30% of the production cost (Liu and Vyverman, 2015). The microalgal cell surface is negatively charged (Henderson et al., 2008a), with cells repulsed from one another and maintained in suspension. Harvesting typically involves dosing a positively charged metal coagulant to neutralise the surface charge allowing the cells to aggregate together creating flocs (Henderson et al., 2008a). Subsequent removal of the flocs is through filtration, sedimentation, centrifugation, or dissolved air flotation (DAF) (Henderson et al., 2008b), with DAF taking advantage of the natural tendency of algae to float by raising the biomass to the surface where it is skimmed off and recovered. Harvesting thorough centrifugation and DAF represent significant chemical and energy costs (Li et al., 2008; Jarvis et al., 2009; Ometto et al., 2014) and require additional assets within the process flow-sheet representing further capital and operational expenditure.

Recommendations to overcome the challenges associated with harvesting suspended cultures include the use of species known to self-flocculate thereby aiding in removal by sedimentation or flotation (Hashimoto and Furukawa, 1989), or alternatively through the selection and growth of a non-suspended/filamentous culture (Liu and Vyverman, 2015). Filamentous species naturally attach together in addition to other particles (i.e. suspended material and other biological entities) forming a biofilm layer on a surface interface (Congestri et al., 2006). This interface can be the surface of the reactor (i.e. floor, walls and baffles), or intentionally submerged substrates e.g. polyurethane and polystyrene foam (Travieso et al., 1996; Ledwoch et al., 2015) used to increase the available attachment surface area. Harvesting is achieved through physically scrapping the attachment surface to remove the biofilm thereby eliminating the costs associated with harvesting suspended biomass (Gross et al., 2015). Non-suspended systems can be further categorised into matrix-immobilisation with microalgal biomass encapsulated in a hydrophilic polymer, whilst reducing the challenges associated with harvesting increases issues related to cellular access to CO_2, nutrients and photons (Moreno-Garrido, 2008).

Such systems are considerably less established than the suspended systems and questions remain around their suitability and affordability for municipal wastewater treatment.

The suspended and non-suspended bioreactors are then further categorised into open and closed systems. Open systems rely on the external environmental conditions to facilitate growth, characterised by the use of solar irradiance with sunlight hours and intensity affecting biomass productivity. Open systems are further influenced by external conditions including temperature; rainfall (instigating culture dilution) and contamination by opportunistic species resulting in variability in annual performance. Alternatively, closed systems contain the biomass within the reactor thereby minimising the opportunity for contamination (Ugwu et al., 2008) and supporting the culture of a mono-community through separation of the biomass from a potentially growth inhibiting environment (Grima et al., 1999). An enclosed system offers greater control of the parameters to optimise growth (Larsdotter, 2006; Ugwu et al., 2008) e.g. irradiance, temperature, evapotranspiration, O_2, CO_2 and pH and encourages increased specific growth whilst requiring greater infrastructure and operational costs limiting their scalability.

The choice of bioreactor is evaluated upon performance at an economically accepted cost (Borowitzka, 1999), with examples including (but not limited to) high rate algal ponds (HRAP), photobioreactors (PBR), attached microalgal biofilms and matrix-immobilisation (Figure 2.2).

Table 2.2 Summary of microalgal bioreactor designs, operating parameters and performance.

Solution	Configuration	Scale	Algal biomass concentration dry mass ($g.L^{-1}$)	Algal community	HRT (d)	References
HRAP	Raceway pond	Full	0.2 – 1.0	Mixed	4 – 10	(Picot et al., 1992; Nurdogan and Oswald, 1995;Christenson and Sims, 2011; García et al., 2000; Craggs et al., 2012)
PBR	Tubular	Pilot	1.0 – 2.0	Mono	1 – 7	(Christenson and Sims, 2011; Di Termini et al., 2011; Ruiz et al., 2013)
	Panel	Lab			2 - 5	
Biofilms	Floway	Full	130 ($g.m^{-2}$)	Mixed	6 – 16	(Davis et al., 1990a; Adey et al., 1993; Craggs et al., 1996a; Wei et al., 2008; Christenson and Sims, 2011;Christenson and Sims, 2012)
	Submerged	Pilot			6	
Matrix-immobilisation	Packed bed	Lab	0.9 – 3.3	Mono or mixed	0.2 – 3	(Chevalier and De la Noue, 1985; Travieso et al., 1996; Filippino et al., 2015)

2.2.1 High Rate Algal Pond (HRAP)

A HRAP is a raceway configured open pond mixed via a paddle wheel to circulate the algal culture and prevent settlement (Hoffmann, 1998; García et al., 2000). Sunlight is the primary method of irradiation and as such, culture depths of 20 – 60 cm are typical (Picot et al., 1992; Borowitzka, 1999; García et al., 2000) to enable optimal light penetration and maximise growth. A HRAP supports a symbiotic community of microalgae and bacteria for the assimilation of nutrients and organic matter (Park and Craggs, 2010) supporting combined microalgal and bacterial concentrations averaging 0.2 g(DW).L^{-1} (Craggs et al., 2012) with maximum concentrations of up to 1 g(DW).L^{-1} reported (Christenson and Sims, 2011). Operational retention times of 4 – 10 days (Picot et al., 1992) are required to enable sufficient contact time with the biomass to achieve the required level of remediation (Table 2.2), resulting in large footprints (Gupta et al., 2015). Of the bioreactors available, HRAPs have received the most attention (Christenson and Sims, 2011) and can be found operational at full scale with a demonstration plant located in New Zealand with individual pond footprints of 1.25 ha (Craggs et al., 2012; Sutherland et al., 2014). Further larger demonstration plants are/will be constructed in California, New Mexico, Hawaii and Florida with the primary focus on biomass production for biofuels (Cai et al., 2013).

2.2.2 Photobioreactor (PBR)

A photobioreactor (PBR) is an example of a closed, suspended system and are available in varying configurations including horizontal or vertical tubular (TPBR) or flat panel reactors (Borowitzka, 1999; Molina et al., 2001; Ugwu et al., 2008; Sierra et al., 2008). A PBR encloses the culture in a series of narrow tubes (e.g. < 4cm diameter (Gupta et al., 2015)) or panels illuminated by sunlight and/or artificial sources (Ugwu et al., 2008). Enclosing the culture enables a greater control of growth conditions, i.e. light, CO_2, O_2 and pH (Christenson and Sims, 2011) and permits the growth of a target species through optimised growth parameters, facilitating an increased biomass concentration in comparison to a HRAP of up to 2.0 g.L^{-1} (Table 2.2) when cultured/remediating domestic wastewater effluents. The culture is circulated through the reactor by pumping and degassing/bubbling processes (additionally releasing excess O_2 produced through photosynthesis). As a consequence of the sophistication of control, PBRs are expensive

to install and operate (Gupta et al., 2015) and are typically only employed in wastewater treatment as a plentiful source of low cost culture medium for growth of a species for the return of a high value product (Cantrell et al., 2008; Gupta et al., 2015) to cover the cost of operation. High biomass production PBRs of up to 4,000 L in capacity are operational and utilised for the cultivation of an inoculum species for HRAPs with the ultimate goal of biofuel production (Cai et al., 2013).

2.2.3 Microalgal Biofilms

Two types of microalgal biofilm processes exist; these include the use of an inclined floway (aka algal turf scrubber) with biofilm attachment to a surface (Craggs et al., 1996a; Craggs et al., 1996b) or submersion of a substrate to support biofilm growth and development (Rectenwald and Drenner, 2000; Wei et al., 2008; Johnson and Wen, 2010) with practical examples including rotating algal biofilm reactors (RABR) (Christenson and Sims, 2012) (Table 2.2). Biomass communities are heterogeneous and multi-layered (Congestri et al., 2006; Kesaano and Sims, 2014) and change seasonally (Hoffmann, 1998) with reported biomass productivity of up to 60.9 $g.m^{-2}.d^{-1}$ (Craggs et al., 1996a) and demonstrating enhanced metabolic activity (Cohen, 2001). Floway systems have been operated at full scale in Florida and California (Adey et al., 1993; Craggs, 2001) with footprints up to 1012 m^2 treating a flow of 109 to 1336 $m^3.d^{-1}$.

2.2.4 Matrix-immobilisation

Matrix-immobilisation is a variant of the attachment theme of reactors through the entrapment of living microalgae cells within a natural or artificial resin (Mallick, 2002). These resins are hydrophilic in nature with small pores to enable the diffusion of wastewater to the entrapped microalgal cells (de-Bashan and Bashan, 2010). Immobilisation enables intensification of a biomass concentration greater than a suspended bioreactor with concentrations up to 3.3 $g.L^{-1}$ reported (Table 2.2). The resin used to immobilise the microalgal biomass can provide additional remediation with the natural resin alginate found to contribute approximately 5% remediation efficiency of ammonium from a synthetic wastewater (Ruiz-Marin et al., 2010) through a chemical bond between the ammonium ions and the carboxyl groups of the resin (Tam and Wong, 2000; Ruiz-Marin et al., 2010). This bond not only removes the ammonium from the source water but concentrations the nutrient for assimilation by the entrapped microalgal

cells (Ruiz-Marin et al., 2010). Further benefits of immobilising a microalgal culture includes the creation of a barrier around the selected species which prevents penetration by other organisms which could inhibit productivity or outcompete the selected species (Moreno-Garrido, 2008; Covarrubias et al., 2012); and following treatment the biomass can be harvested through the low costing option of gravity settlement, eliminating chemical and energy costs associated with suspended systems. However, reduced space for mobility within the matrix leads to high shear stresses with the matrix imposing additional hindrance to photon accessibility as an area of concern along with the cost of the polymeric matrix when considered at full scale (Hoffman, 1998; Zeng et al, 2015). Of the microalgal reactors available, the immobilisation technology for nutrient remediation is within its infancy with the majority of research and knowledge gained to date through lab scale activities with bioreactors of up to 5 L in volume (Tam and Wong, 2000) (Table 2.2, SI Table A.4).

2.3 Influence of Operational Parameters and Bioreactor Design on Remediation Performance

2.3.1 Influent Nutrient Concentration and Treatment Period

The final effluent concentration and treatment period are key criteria when assessing the performance of microalgal bioreactors for wastewater nutrient remediation. European regulations within the Urban Wastewater Treatment Directive (UWWTD) require a final effluent concentration prior to discharge of 15 or 10 $mg.L^{-1}$ total nitrogen (TN) and 2 or 1 $mg.L^{-1}$ total phosphorus (TP) for works treating a population equivalence (PE) of either 10 – 100 k or >100 k respectively (European Community, 1991), with further site specific reductions in P to ~0.1 $mg.L^{-1}$ proposed with the onset of the water framework directive in 2015. Furthermore, the inclusion of an additional asset within a flow sheet to satisfy the required discharge concentrations must complement upstream processes to enable a constant output and flow. Microalgal bioreactors have been analysed for the treatment of a variety of wastewater streams including primary and secondary domestic effluent, dairy manure wastewater, agricultural run-off and centrate (SI, Appendix A) with remediation data available for a wide range of influent concentration from 3.3 – 309 $mg.L^{-1}$ for ammonium and 0.04 – 770 $mg.L^{-1}$ for phosphorus (Figure 2.3).

When comparing microalgal bioreactor options for ammonium remediation, the influent and effluent concentrations show no clear relationship (Figure 2.3a) with effluent concentrations ranging from 0.11 – 140.9 $mg.L^{-1}$. Those showing enhanced remediation performance include a TPBR with a 99.7% removal efficiency and effluent concentration of 0.11 $mg.L^{-1}$ (Di Termini et al., 2011), in addition to a polystyrene submersion system achieving 99.9% removal and an effluent concentration of 0.3 $mg.L^{-1}$ (Johnson and Wen, 2010) (Figure 2.3a). These systems were either inoculated or naturally dominated by species of chlorophyceae well known for their nutrient remediation abilities, namely *Chlorella* sp. (TPBR) and *Scenedesmus* sp. (biofilm). Both systems demonstrated high biomass yields and productivities with a specific growth rate of 0.39 d^{-1} for *Scenedesmus* sp. and an increase in biomass concentration from 0.4 to 2 $g.L^{-1}$ (approximate maximum biomass concentration reported for a TPBR configuration (Christenson and Sims, 2011)) and *Chlorella* sp. with a biomass concentration of 30 – 35 $g(DW).m^{-2}$ equivalent to a productivity of 2 – 4 $g.m^{-2}.d^{-1}$ (Johnson and Wen, 2010).

Those bioreactors which did not perform as well belonged to the biofilm category of reactors with an algal turf scrubber demonstrating a 24.2% removal and effluent concentration of 2.5 $mg.L^{-1}$ (Craggs et al., 1996b) and a PBR containing rough surfaces to facilitate biofilm attachment with a removal efficiency of 45.8 % and an effluent concentration of 26.0 $mg.L^{-1}$ (Karapinar Kapdan and Aslan, 2008). Although the algal turf scrubber reported an extremely high yearly biomass productivity of 35 $g.m^{-2}.d^{-1}$, the biofilm contained a significant proportion of bacterial matter, particulates and cyanobacteria with the chlorophyceae *Chlorella* sp. and *Scenedesmus* sp. reported as only ‘present’ or ‘few’ (Craggs et al., 1996b). Findings from these studies demonstrate the importance of species selection in addition to biomass concentration for the enhanced remediation of ammonium. Performance could therefore be improved by an increased biomass concentration or longer contact time with the available biomass to facilitate remediation through the direct mechanisms.

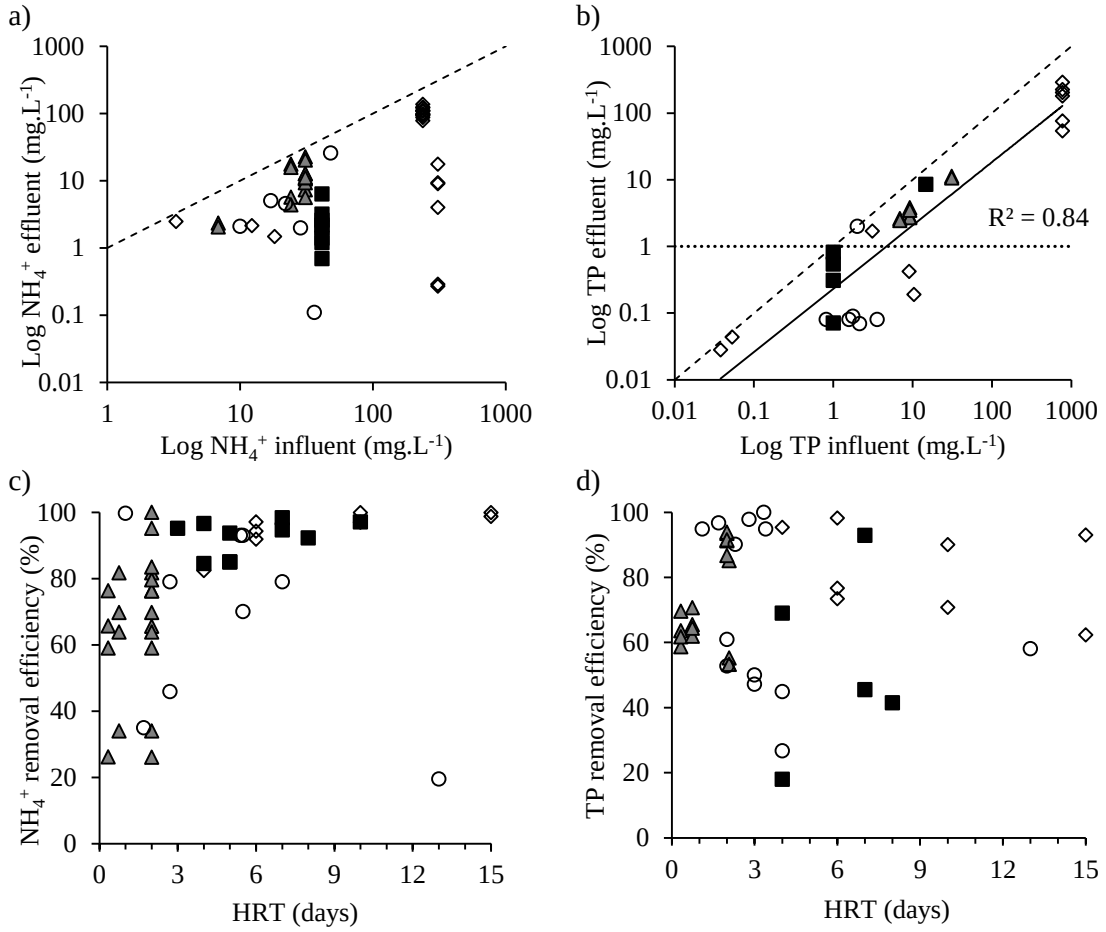

Figure 2.3 Influent concentration vs effluent concentration for a) ammonium and b) phosphate; and treatment period vs remediation efficiency for c) ammonium and d) phosphate for all bioreactors. HRAP (■), biofilms (◊), matrix-immobilisation (▲) PBR (○) and parity line (--).

Whereas for phosphorus, a reasonable log-log relationship ($r^2 = 0.84$) is observed for all bioreactors (Figure 2.3b) with a lower influent concentration resulting in lower effluent concentration despite vast differences in operating parameters including biomass concentration, treatment time and irradiance. This relationship suggests that unlike ammonium, mechanisms other than direct remediation are primarily responsible for the remediation of phosphate with treated effluent concentrations of < 1mg.L^{-1} possible at influent concentrations < 10 mg.L^{-1} (Figure 2.3b) providing there is an adequate supply of nitrogen (Beuckels et al., 2015).

In terms of treatment period, performance data for NH_4-N removal exhibits a more defined relationship with performance efficiency and treatment period, in comparison to

PO_4-P (Figure 2.3c and d). The enclosed and more intensive reactors remediate > 80% NH_4-N within less than 2 days (Figure 2.3c) with HRTs > 3 days required by HRAPs through the necessary increased contact period with the reduced biomass concentration. The biofilm solutions are characterised by a high biomass concentration but require a HRT > 10 days for a > 90% NH_4-N removal (Johnson and Wen, 2010), however this is a necessary design feature as increasing the flow velocity (hence reducing the HRT) creates increased shear stress reducing biofilm colonisation (Roeselers et al., 2008) with impacts on remediation performance and increased suspended solids within the treated effluent

In comparison, PBRs and matrix-immobilisation can achieve > 99% NH_4-N remediation efficiency within HRT < 2 days through an increased biomass concentration and protection from biomass washout with increased flows. For example, a matrix-immobilised system with an algal concentration of 10^6 cells.bead^{-1} and 11.7 beads.mL^{-1} was shown to remediate 100% NH_4-N within 24 h (Tam and Wong, 2000), in addition to a TPBR with a 2 g.L^{-1} biomass concentrations achieving a 99.7% NH_4-N remediation (Di Termini et al., 2011). The remediation of ammonium is a function of biomass concentration and contact time, with those bioreactors with a dilute biomass concentration requiring a greater treatment period in comparison to bioreactors with high biomass concentrations, with biofilms an exception through operational limits.

A relationship between HRT and TP removal efficiency is not as clear as NH_4-N (Figure 2.3d) further supporting previous assumptions that an alternative mechanism other than direct remediation significantly contributes to the removal of phosphate. Phosphorus removal efficiencies are generally <69% for HRAPs with removal of approximately 40% seen in the majority of studies (SI, Table A.1). The cases where >90% removal are observed are through the modification of parameters to enhance the performance of a HRAP for phosphorus removal. For example, a 99% PO_4^{3-} removal efficiency and a residual of 0.07 mg.L^{-1} (SI, Table A.1) through the addition of lime (CaO) to promote autoflocculation and the precipitation of phosphorus (equivalent to the indirect mechanism) (Nurdogan and Oswald, 1995), with a consensus that the role of indirect removal plays a more significant role than that of direct in HRAPs systems (Mesplé et al., 1996; García et al., 2000;).

2.3.2 Light, Temperature and Biomass Productivity

As microalgae are photosynthetic organisms, metabolic processes associated with nutrient assimilation through growth are driven by light (Grima et al., 1999; Janssen et al., 2003); with light described as a key parameter of microalgal reactors (Grima et al., 1999; Janssen et al., 2003; Ugwu et al., 2008). The required light intensity for optimal growth is species specific with an example range of 150 – 400 $\mu mol.m^{-2}.s^{-1}$ reported for *Scenedesmus* sp. (Liu et al., 2012; Gris et al., 2014). Light intensities below a species threshold range are associated with a reduction in biomass productivity (Gris et al., 2014) and can be generated through light limitation as a result of high density microalgal cultures creating self-shading and/or light attenuation and reduction with an increasing transmittance pathway (Arbib et al., 2013). Intensities beyond a species' preferred range results in oxidative damage through photoinhibition associated with a reduction in biomass productivity with the dissipation of the excess photons into heat (Gordon and Polle, 2007). To illustrate, Di Termini et al, (2011) observed a specific growth rate of 0.39 d^{-1} and a remediation efficiency of >98% for NH_4-N and PO_4-P for an autochthonous culture of *Scenedesmus* sp. when grown within an indoor TPBR with a constant light intensity of 200 $\mu mol.m^{-2}.s^{-1}$ in comparison to a reduced growth rate of 0.02 d^{-1} and < 80% NH_4-N and PO_4-P removal efficiency within an outdoor TPBR with a variable light intensity reaching a daylight maximum of 1,300 $\mu mol.m^{-2}.s^{-1}$.

As a consequence of biomass concentration, incident light intensity and culture depth (hence light transmittance depth), multiple 'light zones' are simultaneously evident within microalgal bioreactors (Ogbonna and Tanaka, 2000). These zones can be described as light inhibited, saturated, limited and no light with the zones determined by the increasing depth from the culture surface, with a changing profile as a consequence of the incident light intensity and biomass concentration (Kumar et al., 2015) estimated through the Beer-Lambert Law (Grima et al., 1999; Lee, 1999). The challenge of microalgal bioreactors is to maintain the culture within the light saturating zone to enable optimal productivity and the associated direct remediation of the target nutrients. This can be achieved through 1) reducing the light transmittance pathway (short depths) (Gupta et al., 2015), 2) increasing culture circulation through mixing to ensure the microalgal cells move within the saturation zone (Sutherland et al., 2014) and 3) maximising the surface

to volume ratio (Janssen et al., 2003; Christenson and Sims, 2011; Arbib et al., 2013) to ensure sufficient light reaches the culture surface.

Outdoor systems are typically exposed to variable levels of light intensities through seasonal (and daily) changes in the available solar radiation. Intensities during the summer months of >1,200 $\mu mol.m^{-2}.s^{-1}$ in comparison to 170 - 685 $\mu mol.m^{-2}.s^{-1}$ are reported in the winter months (SI, Table A.1). However, only 50% of the radiation provided by sunlight is available to the microalgae for use as photosynthetically active radiation (PAR) (400 – 700 nm) (Walker, 2009) with open systems demonstrating poor photosynthetic efficiency in the conversion of solar energy into chemical energy of approximately 1.5% (Norsker et al., 2011). The variability in light intensities are reflected in fluctuating biomass productivities with ranges between 4.4 – 11.5 $g.m^{-2}.d^{-1}$ observed in a 5 ha demonstration HRAP plant with removal efficiencies for NH_4-N and dissolved reactive phosphorus (DRP) between approximately 40 – 80% and 10 – 50% respectively, mirroring the pattern in seasonal biomass productivity (Craggs et al., 2012).

Artificially lit reactors are employed to overcome the variability in biomass productivities and the associated treatment profiles, with generally lower intensities in the range of ~ 200 – 400 $\mu mol.m^{-2}.s^{-1}$ reported for commercial photobioreactors (Gordon and Polle, 2007). Exposure to lower intensities are possible through design optimisation (e.g. surface to volume ratio) to enable effective use of the provided light with increased photosynthetic efficiencies of 3 – 5% for PBRs (Norsker et al., 2011) and/or the selection of a light source with a specific wavelength within the PAR to enable a more efficient use of the provided light, particularly as light is supplied at an operational cost. For instance, within PAR the microalgal chlorophyll molecules absorb light more efficiently within the blue (~400 nm) and red (~600 – 700 nm) region of the spectrum, with exposure to these wavelengths improving the photosynthetic efficiency and enhancing biochemical processes aligned to nitrogen and phosphorus remediation. For example, growth under a blue light regime is associated with increased phosphorus remediation through the activation of protein synthesis (Figueroa et al., 1995), demonstrated by a culture of *Scenedesmus* sp. with a 45% increase in removal rate under a blue light regime of 1.8 $mg.L^{-1}.d^{-1}$ in comparison to 1 $mg.L^{-1}.d^{-1}$ when grown under white light (400 – 700 nm) (Kim et al., 2013). Furthermore, red light is known to enhance microalgal growth rate,

with a 38% increase in the specific growth rate of a culture of *Spirulina platensis* in comparison to growth of the same species within white light (Wang et al., 2007).

The use of constant artificial light can represent a significant proportion of the total operational costs. Strategies are employed to improve the efficiency of artificial light which can be reflected within these costs and include (as discussed) the selection of an appropriate intensity to minimise wasted photons and the application of a light source with a suitable wavelength (i.e. LEDs) to eliminate energy use on unutilised wavelengths (Yeh and Chung, 2009). However, the antenna structure of the microalgal light harvesting complex is unable to absorb all the photons provided under constant light (Park and Lee, 2000) offering a further option of cost reduction and increased photosynthetic efficiency through reduced photoperiods and flashing/pulsating light regimes. For instance, under a flashing light regime of 37 kHz the cell concentration of a culture of *C. vulgaris* was 20% greater than that of the same species grown under a constant light regime (Park and Lee, 2000).

Overall, artificial lighting offers a variety of options for increasing biomass productivity and associated remediation of nutrients through lighting regimes, which cannot be benefitted from within open systems. Advances made within LED industry resulting in increased bulb life, associated energy savings and predicted reduction in unit cost over time (Ibrahim et al., 2014) makes the use of artificial lighting a more attractive option for intensifying the remediation performance of microalgal reactors.

Microalgae exhibit a similar relationship to temperature as light, profiled by an increase in biomass productivity (and associated nutrient remediation) with increasing temperature (Singh and Singh, 2015) until reaching a critical temperature, beyond which has a negative effect on growth. For instance, Martinez et al. (1999) documented an increase in specific growth rate of 0.69 d^{-1} to 1.10 d^{-1} for *Scenedesmus* sp. grown within secondary wastewater effluent with an increasing temperature from 20 to 30^{o}C coupled with >90% remediation of nitrogen and phosphorus. At 35^{o}C, the specific growth rate decreased to 0.46 d^{-1} with a remediation efficiency of 79% and 54% for nitrogen and phosphorus respectively. A temperature range between 15 – 30^{o}C (Larsdotter, 2006; Singh and Singh, 2015) are believed optimal for microalgal bioreactors, with maximum critical

temperatures species specific, providing the external nutrient concentration and light provision are not limiting (Singh and Singh, 2015).

Maintenance of a constant temperature is challenging within open reactors (Singh and Singh, 2015) with seasonal variations from 7.2 to 25^{o}C documented (SI, Table A.1) and extreme lows of 5^{o}C and highs >30^{o}C reported for HRAPs (Nurdogan and Oswald, 1995). Application of open systems are therefore favoured within locations with suitable annual climates to facilitate biomass productivity and achieve the required level of remediation throughout the year; for example a floway periphyton scrubber located in the Florida Everglades with a daily mean air temperature of 19^{o}C corresponding to a water temperature ranging from 18.1 – 27.2^{o}C (Adey et al., 1993). Furthermore, microalgae within low temperature environments are more susceptible to photoinhibition which can constrain the use of open reactors in countries with a cold climate (Larsdotter, 2006), particularly with winter light intensities of up to 600 $\mu mol.m^{-2}.s^{-1}$ reported (García et al., 2000). Temperature maintenance in closed reactors (and systems located indoors) are easier to control with documented temperatures of 20 – 30^{o}C (SI, Table A.2 - Table A.4), corresponding to the range of temperatures associated with enhanced growth and nutrient remediation and more suited to locations with a cooler annual climate.

2.4 Conclusions and Key Remaining Challenges

Algal treatment of wastewater, realised through a combination of direct uptake and indirect removal associated with elevated pH provides a potential alternative to traditional tertiary treatment options for nutrient removal. Recent advancements in the understanding of both the mechanisms by which algal remediate nutrients in wastewater and specifically non-suspended algae treatment systems attests to the suggestions outlined by Hoffman, (1998). Accordingly, the ability of algal based wastewater treatment to meet future challenges can be viewed with greater confidence. The most pressing illustration of which is associated with compliance to emerging sub 1 $mg.L^{-1}$ phosphorus discharge standards. Further, developments in non-suspended systems have significantly reduced the required HRT of such systems to mirror existing passive tertiary treatment technologies. Consequently, consideration of the use of microalgal treatment can more reliably extend to sites where previously the lack of availability of sufficient inexpensive land was seen as a barrier to uptake of high rate algal ponds.

Future research will likely focus on the remaining challenges that require resolution before widespread use of algae can be realised. The costs associated with either harvesting of suspended systems, irradiance of closed systems and/or the chemicals associated with either harvesting and matrix-immobilisation require better understanding and optimisation to truly established the relative merit of microalgal systems compared to alternative tertiary treatment systems. As part of that better refinement is the understanding of the added value algae systems can offer (in terms of associated bioenergy production, biofuels and bio products). This will become increasingly important in positioning microalgae treatment options as part of the delivery of the circular economy, which is expected to increasingly shape future investment consideration. Furthermore, the associated removal of hazardous chemicals and the ability for total nutrient removal need exploring in detail so that they can be properly valued. In addition, technical challenges remain associated with intensification and seasonal stability (HRAP), scalability and light utilisation (PBRs and non-suspended) and selection of better strains/mixtures to match the target wastewater and maximise biomass growth and byproduct yields (all systems). Whilst development and increased uptake of all reactor types should be expected it is perhaps in relation to non-suspended systems that the greatest advancements can be anticipated. Increasing demonstration of non-suspended systems will better enable appropriate comparison to be made with HRAP and PBRs and practical optimisation achieved. Ultimately this will enable the potential for such systems to be considered in places where HRAPs are either not practical or desirable such as small wastewater treatment works with limited land availability.

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Chapter 3. Influence of Microalgal N and P Composition on Wastewater Nutrient Remediation

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Abstract

Microalgae have demonstrated the ability to remediate wastewater nutrients efficiently, with methods to further enhance performance through species selection and biomass concentration. This work evaluates a freshwater species remediation characteristics through analysis of internal biomass N:P (nitrogen:phosphorus) and presents a relationship between composition and nutrient uptake ability to assist in species selection. Findings are then translated to an optimal biomass concentration, achieved through immobilisation enabling biomass intensification by modifying bead concentration, for wastewaters of differing nutrient concentrations at hydraulic retention times (HRT) from 3 h to 10 d. A HRT <20 h was found suitable for the remediation of secondary effluent by immobilised *Scenedesmus obliquus* and *Chlorella vulgaris* at bead concentrations as low as 3.2 and 4.4 bead.mL^{-1}. Increasing bead concentrations were required for shorter HRTs with 3 h possible at influent concentrations < 5mgP.L^{-1}.

Keywords: microalgae, internal composition, species, biomass, immobilisation

3.1 Introduction

Microalgae are photosynthetic organisms that assimilate nitrogen (N) and phosphorus (P) during their growth. The subsequent biomass generated can be converted into energy or further raw materials following appropriate processing (Ometto et al., 2014), offering benefits in its use and renewing interest in a microalgae based technology for wastewater nutrient remediation.

Nutrient remediation characteristics for N and P have been shown to positively correlate to growth rate (Xin et al., 2010) with growth a function of internal rather than external nutrient concentration (Portielje and Lijklema, 1994). The internal composition of marine phytoplankton has been established as 106:16:1 as a molar ratio for C:N:P, known as the Redfield Ratio (Redfield, 1934). However, in the case of freshwater microalgae, the Redfield Ratio is an exception rather than a rule with N:P molar ratios ranging between 8:1 and 45:1 (Hecky et al., 1993) through a species' specific cellular quota for structural components and storage for growth (Droop, 1968). More importantly, freshwater microalgae have been shown to be able to adjust the N and P concentration in their biomass in relation to the surrounding concentration in the water (Beuckels et al., 2015; Choi and Lee, 2015) with biomass P accumulation influenced by the external P and N supply whereas N accumulation is independent of P (Beuckels et al., 2015). This behaviour is due to the predominate use of nitrogen for protein synthesis with P incorporated into ribosomal RNA. Accordingly, under limited nutrient conditions cell growth is reduced whilst carbon uptake continues (through photosynthesis) resulting in enrichment of carbohydrates or lipids. This is often exploited prior to bioenergy recovery to maximise yield for the microalgae biomass (Craggs et al., 2013). In high nutrient environments, microalgae can also accumulate excess nutrients through luxury uptake pathways (Eixler at el., 2006) enabling adaptation across a wide range of environmental situations. Such flexibility in nutrient compositions enables microalgae to successfully adapt to the local environment and influences the biochemical composition of the resultant biomass (Loladze and Elser, 2011, Choi and Lee, 2015).

Furthermore, the nutrient remediation characteristics of microalgal species have been correlated to the internal elemental concentration, with P remediation inversely correlated to biochemical composition (Choi and Lee, 2015; Ruiz-Martínez et al., 2015). With the

nutrient concentration in microalgal biomass shown to vary significantly from 0.03 – 3% of dry mass for P and between 3 – 12 % for N (Reynolds, 2006), the design of microalgae reactors for wastewater treatment based on fixed stoichiometry (Redfield Ratio) are not likely to be reliable. Studies to date have analysed the impact of varying N:P mediums on cell composition, or evaluated a suitable wastewater nutrient balance for microalgal treatment in relation to internal composition for a specific species (Choi and Lee, 2015). It is posited however, that the efficacy of nutrient remediation can be further enhanced through a targeted selection of a species with a suitable composition following adaptation to a balance of nutrients in a wastewater to be processed, thereby achieving an enhanced level of remediation.

Furthermore, the majority of the work to date on microalgal wastewater nutrient remediation has considered suspended microalgal biomass operated in relatively passive technologies such as high rate algae ponds (HRAP). HRAPs are typically configured as raceways ponds with shallow depths (20 – 60 cm) containing dilute biomass concentrations of microalgae and bacteria of approximately 0.2 g(DW).L^{-1} (Craggs et al., 2012). Biomass concentration is relatively low through the variability of the light source (solar radiation) and associated poor light efficiency, in addition to other external factors related to open systems including temperature, predation and contamination (Park et al., 2011). Consideration and uptake of microalgae based technology for wastewater treatment is restricted in many countries due to the large footprints associated with the required long HRTs and shallow depths (Lundquist et al., 2010). Intensification of the algae biomass (and reduction in footprint) can be achieved through immobilisation where the biomass is encapsulated within an alginate gel affording biomass concentrations of up to 3.3 g(DW).L^{-1} (Chevalier and De la Noue, 1985). Whilst the technology is within its infancy, remediation of PO_4-P and NH_4-N from secondary wastewater effluents from 1.1 mgP.L^{-1} and 2.6 mgN.L^{-1} to 0.07 mgP.L^{-1} and 0.02 mgN.L^{-1} have been demonstrated for immobilised *S.obliquus* within hydraulic retention times of 6 h (Whitton et al., 2014). In addition to a concentrated biomass and reduced HRT, immobilisation facilitates the removal of biomass post-treatment through gravity settlement; eliminating costs associated with harvesting technologies which require coagulation and intensive energy requirements i.e. centrifugation. Following these positive attributes, the immobilised technology warrants further research to determine whether the solution can be optimised

for adequate treatment within suitable HRTs prior to further development to improve its suitability for application within the wastewater treatment industry e.g. operational costs related to bead longevity and resin material.

Immobilisation affords the ability to seed and maintain a chosen species or community with known nutrient removal capacities such that it is posited that appropriate bead concentrations can be tailored to the required loading rates. To date, the work completed to optimise biomass density through bead concentration have pre-selected a bead.mL^{-1} concentration and evaluated remediation performance regardless of the chosen species nutrient uptake characteristics. For example, Abdel Hameed (2007) evaluated the remediation performance of *Chlorella vulgaris* using bead concentrations of 10.66, 16, 32 and 64 bead.mL^{-1} (1:3 to 2:1 bead:wastewater v/v) at 10^6 cells.bead^{-1}. Concentrations of 10.66 and 16 beads·mL^{-1} both achieved 100% NH_4^+ and 95% PO_4^{3-} removal efficiency, suggesting a concentration of 10 beads.mL^{-1} and associated biomass concentration to be suitable for optimal treatment under the conditions tested, with the possibility of a lower concentration performing similarly.

With the onset of the water framework directive (WFD) across Europe, the discharge consent for wastewater P will reduce from the current 1 – 2 mgP.L^{-1} outlined within the Urban Wastewater Treatment Directive (UWWTD) to <0.5 mgP.L^{-1}, with some sites expected to be as low as 0.1 mgP.L^{-1} (Mainstone and Parr, 2002; Jarvie, 2006). Microalgae can be considered an alternative solution to meet these new stringent targets, providing the solution represents a practical alternative in terms of treatment time (HRT) and footprint, which can be achieved through immobilisation.

As such, the objectives of this study are to investigate how the internal composition of microalgae, through their ability to adapt to the external nutrient concentrations, relate to their nutrient uptake. Remediation performance of two of the characterised species, *C.vulgaris* and *S.obliquus*, are further analysed within real wastewater effluent. The findings are then translated into the impact on the design of an immobilised reactor for the improved remediation of wastewater nutrients; through the selection of a species for immobilisation and manipulation of biomass concentration through bead concentration to enable a suitable HRT for the integration of a microalgal reactor into a wastewater flow sheet for nutrient polishing.

3.2 Materials and Methods

3.2.1 Microalgal biomass culture and immobilisation

The freshwater species *Chlorella vulgaris* (211/11B), *Chlorella sorokiniana* (211/8K), *Microcystis aeruginosa* (1450/3), *Scenedesmus obliquus* (276/3A) and *Stigeoclonium* sp (477/24) were obtained from the Culture Collection for Algae and Protozoa (CCAP) (Oban, UK). Mono cultures were cultivated in 100 L reactor containing 50 L of medium as recommended by CCAP for optimal growth (Supplementary information, Appendix B) with an N:P molar ratio of approximately 2:1 for *M.aeruginosa* and 6:1 for the remaining species (SI, Appendix B). Cultures were illuminated under a 24 hour light regime with a light intensity of approximately 100 – 150 $\mu mol.m^{-2}.s^{-1}$. Constant mixing was through a circulation pump (900 $L \cdot h^{-1}$) (Hydor Koralia Nano 900), with no external supply of CO_2 provided and the temperature maintained at 18^{o}C. Microalgal biomass was harvested prior to the onset of stationary growth phase determined through previous growth experiments to characterise growth under the stated operational conditions and monitored through; cell counts for single celled species using a haemocytometer and light microscope (Olympus, BH Series), or dry weight following standard methods for total suspended solids (TSS) (APHA, 2005) for filamentous species. Knowledge of the chosen species' growth profile enabled biomass harvesting at the latter stages of exponential growth.

Microalgal immobilisation and encapsulation within calcium-alginate beads were completed following the method of Ruiz-Marin et al., (2010), with the adsorption capacity of the calcium-alginate resin determined through the method of Gotoh et al. (2004) (SI, Appendix C).

3.2.2 Freshwater species characterisation - nutrient remediation and internal N and P composition

Nutrient removal batch trials were completed in 100 L reactors using 50 L modified BG11 medium (SI, Appendix B.2) under the same operational conditions as those for cultivation and supplemented with NH_4Cl and KH_2PO_4 for an N:P molar concentration of 2:1, selected as a N:P < 10:1 is associated with enhanced biomass productivity (Choi and Lee, 2015). Biomass was seeded at an approximate concentration of 40 $mg(DW).L^{-1}$. Reactors

were mixed on a daily basis and pH monitored and corrected to pH 7 using 1 M NaOH and HCl to prevent alkalisation of the medium and nutrient remediation through the indirect processes of precipitation and volatilisation. Batch trials were run over a period of 10 days, with analysis on day 0, 3 5, 7 and 10.

Nutrient remediation was determined by measuring the residual concentration of NH_4-N and total phosphorus (TP) within the medium in triplicate using Spectroquant test kits1.14752.0001 (NH_4-N) and 1.14543.0001 (PO_4-P) (Merck Millipore), following the manufacturer's instructions and read via a Spectroquant Nova 60 spectrophotometer. Biomass growth was monitored as previously described and specific growth rate calculated using Equation 3-1, where μ = specific growth rate (d^{-1}), $x1$ and $x2$ the biomass concentration in cells.mL^{-1} or mg(DW).L^{-1} at time t1 (d) and t2 (d).

$$\mu = \frac{\ln(\frac{x1}{x2})}{t2 - t1} \qquad \text{Equation 3-1}$$

Characterisation of the internal total nitrogen (TN) and C content of the microalgal biomass was analysed following freeze drying (ModulyoD Freeze Dryer, USA) and analysis using a TCN Vario III Elemental Analyser (Isoprime, DE) according to standard method ISO 10694:1995. Phosphorus content of the digested biomass sample was measured by UV/VIS spectrophotometry, following calibration with P standards of 0 – 7 mg.L^{-1} with a ± 0.005 accuracy, according to standard methods (USEPA, 1995).

3.2.3 Wastewater nutrient remediation trials for *S.obliquus* and *C.vulgaris*

Secondary wastewater effluent was delivered weekly from a wastewater treatment works located in the Midlands, UK and stored at 4°C until use. The 32,000 population equivalence (PE) wastewater treatment plant (WWTP) comprises of an oxidation ditch operated for biological nutrient removal in addition to iron salt precipitation prior to the secondary clarifier. Effluent was selected from this site as the WWTP is located in a catchment designated as a Site of Special Scientific Interest (SSSI) which will be required to meet the stricter P consents prescribed within the WFD. As such, the WWTP has been selected as a trial site to evaluate the performance of multiple alternative technologies for the purpose of P polishing to meet the upcoming change in consent.

The average characteristics of the effluent collected were 0.3 mg.L^{-1} PO_4-P and 0.1 mg.L^{-1} NH_4-N. Effluent was supplemented with NH_4Cl and KH_2PO_4 to compensate for the current dosing strategy (which ensures appropriate nutrient discharge to the SSSI) and maintain a set NH_4-N concentration of 5 mg.L^{-1} and a range of PO_4-P concentrations between 0.5 and 10 mg.L^{-1}. These concentrations represent a possible range of secondary effluent characteristics that could be encountered by a tertiary microalgal system without advanced upstream treatment (A. Brookes & P. Vale, 2011, pers. comms., 20 October), with an N:P molar ratio between approximately 22.1 to 1.1 suitable for microalgal activity with ratios < 22 indicating sufficient phosphorus (Hecky et al., 1993). The wastewater also contained a non-supplemented and variable NO_3-N concentration of a maximum of 2 mgN.L^{-1}, lower than the concentration of NH_4-N, which was not analysed through the preference of microalgae to assimilate NH_4-N over NO_3-N (Lau et al., 1995); and results from previous trials which found no accumulation of NO_3-N associated with the nitrification of NH_4 but rather a decrease of NO_3-N in parallel to NH_4-N remediation.

Conical flasks with 250 mL modified effluent were seeded with 10^4 and 10^5 cells.mL^{-1} of *S.obliquus* and *C.vulgaris* respectively to ensure a sufficient initial biomass concentration for growth. Batch trials were run over a period of 7 days under the same operational conditions as those for cultivation with sample analysis on days 3, 5 and 7. Analysis included pH, NH_4-N, PO_4-P and cell concentration. Residual nutrient concentrations and cell concentration were analysed as previously described.

The NH_4-N and PO_4-P cell uptake rate for *S.obliquus* and *C.vulgaris* was estimated through the analysis of the residual concentration according to Equation 3-2, where V is the cell uptake rate (mg.$cell^{-1}$.d^{-1}), N the cell concentration (cells.mL^{-1}) at time t (d^{-1}) and C_i and C_f the initial and final residual concentrations (mg·L^{-1}) respectively.

$$V = \frac{C_i - C_f}{N\ x\ t} \qquad \text{Equation 3-2}$$

3.2.4 Calculations for optimal biomass concentration for wastewater treatment by *S.obliquus* and *C.vulgaris* – suspended and immobilised cultures

Following determination of the cell uptake rate for *S.obliquus* and *C.vulgaris*, calculations were completed to determine initial biomass concentrations required for 'optimal

remediation' of phosphorus (residual <0.1 mgP.L^{-1}) in line with changes to P discharge consent within the forthcoming WFD. Results were translated to an immobilised culture assuming a fixed cell stocking of 10^6 cells.$bead^{-1}$ as recommended by Abdel Hameed, (2007). Calculations estimated the biomass concentration required for the remediation of phosphate at concentrations of 1, 5, and 10 mgP.L^{-1} operating at a range of HRTs varying from 3 h to 10 days. The range of HRTs chosen complement the work by Whitton et al., (2014) which characterised remediation of an immobilised system at HRTs of up to 20 h and compared to the typical retention time of a HRAP (4 – 10 days).

3.3 Results

3.3.1 Freshwater species characterisation - nutrient remediation and internal N and P composition

3.3.1.1 N:P composition changes with change in external N:P

Following cultivation, the internal molar N:P composition of the tested algal species ranged from 7.8 to 20.3 (Table 3.1) despite the similar N:P molar concentration (6:1) of the growth medium (excluding the medium for *M.aeruginosa,* (2:1)), demonstrating the potential significance of algal selection.

Table 3.1 Specific growth rate and internal N:P composition prior to and after nutrient remediation trials, mean (±standard error).

Species	Specific growth rate (d^{-1})	Start (Day 0)		Day 3 - 10	End (Day 10)	
		N ($\mu g.\mu g^{-1}$)	P ($\mu g.\mu g^{-1}$)	N:P (molar)	N ($\mu g.\mu g^{-1}$)	P ($\mu g.\mu g^{-1}$)
		N:P (molar)			N:P (molar)	
Stigeoclonium sp.	0.19 (0.03)	66.0 (0.6)	7.72 (0.1)	13.5 (0.6)	46.8 (0.3)	7.3 (0.1)
		18.9 (0.2)			13.9 (1.3)	
C.vulgaris	0.17 (0.04)	69.8 (0.4)	9.2 (1.2)	10.5 (0.9)	75.6 (2.9)	24.0 (11.8)
		16.8 (1.3)			12.0 (1.2)	
S.obliquus	0.10 (0.03)	79.5 (2.1)	22.6 (2.8)	9.6 (0.5)	81.5 (7.8)	16.0 (3.0)
		7.8 (0.8)			11.3 (2.2)	
C.sorokiniana	0.12 (0.05)	82.4 (17.8)	9.8 (0.8)	15.0 (1.0)	75.4 (2.7)	24.5 (11.0)
		20.3 (1.1)			16.3 (0.8)	
M.aeruginosa	0.06 (0.03)	87.0 (nd)	11.6 (nd)	16.6 (1.0)	91.0 (nd)	13.3 (nd)
		16.6 (nd)			15.1 (nd)	

nd = undetermined

Transferring the algal species from the growth medium to a more N limited test medium (N:P 2:1) for the nutrient remediation trials, reduced the difference in the biomass nutrient molar ratio to between 11.3 and 16.3 (average N:P 13.7) (Table 3.1). The molar N:P composition of *M.aeruginosa* was found to change the least, with a decrease of 1.5 from a molar N:P of 16.6 to 15:1 due to the similarities in the N:P characteristics of the cyanobacteria growth medium and test medium (both N:P 2:1) (Table 3.1). The difference observed with the change in N:P medium is congruent with the microalgae adapting the nutrient concentration within its biomass to the new environment (Beuckels et al., 2015), with the nitrogen content varying between 6.5 and 9.0% by weight (0.065 and 0.09 mgN.mg biomass^{-1}) with comparable variations in biochemical N composition of *Chlorella* sp of 3.6 to 10% previously demonstrated (Åkerström et al., 2014).

All microalgae were found to adjust their internal N:P content within the first 3 days of the trial and remained at their new N:P composition for the remainder of the trial (Table

3.1). For example, *Stigeoclonium* sp. demonstrated a change from an initial N:P of 18.9 (± 0.2) to an average of 13.5 (±0.6) for days 3 – 10 of the experiment, concluding with an N:P of 13.9 (±1.3) by day 10 with mass balances estimating 94.0 and 93.9% of N and P removal through microalgal growth. The final internal N:P values exhibited by all the microalgae analysed generally decreased (excluding *S.obliquus*) through the adjustment of the microalgae.

Whereas species with higher initial N:P composition (>16) reduced to between 12.0 and 16.3, *S.obliquus* with the lowest initial N:P of 7.9 increased to 11.6 (Table 3.1) suggesting *S.obliquus* can tolerate a greater N concentration than supplied by the growth medium (N limited) for incorporation and conversion into new biomass, supporting previous work of tailoring growth mediums to species' biochemical composition for growth optimisation (Mandalam and Palsson, 1998).

An average phosphorus content of 0.01 mgP.mg biomass^{-1} (ranging 0.8 – 2.1% by weight) (Table 3.1) was found for all species, characterising growth in non-limiting P conditions (Hessen et al., 2002). The narrow variation in P within the biomass of the different algae is consistent with previous work that has shown that P level vary less when the N content within the biomass is relatively low (Beuckels et al., 2015). The N content therefore dictated the overall N:P composition of the species (varying from 0.07 – 0.09 mgN.mg biomass^{-1}), and remained within the N:P ranges of 8.5 – 42 and 4.1 – 32 previously reported for *C.vulgaris* and *S.obliquus* (Rhee, 1974; Oh-Hama and Miyachi, 1988, Beuckels et al., 2015) when grown within varying N:P concentrations of differing retention times and growth conditions.

3.3.1.2 Nutrient remediation performance and internal N:P composition

Analysis of the remediation through a reduction in liquid phase nutrient concentration revealed > 99% removal of NH_4-N during the experimental period with species with an initial N:P >18 compared to between 24.7 and 60.8% for species with an N:P <18. The increase in uptake by species characterised with a greater N composition is associated with the greater nitrogen content required per cell supporting previous work demonstrating a relationship between cell growth rate (Droop, 1974) and N remediation characteristics (Choi and Lee, 2015) in relation to internal concentration of the algal cell. The remediation efficiency of phosphate was lower than ammonium, at between 12.5 and

19.6% and unlike N, species characterised as more P limited were found to remediate at the higher end of this range supporting the inverse relationship demonstrated by Ruiz-Martínez et al., (2015) for P uptake and biomass composition.

Mass balances considering biomass growth and N:P composition estimates between 82.6 – 94.5% and 83.7 – 98.1% of N and P remediation is attributed to incorporation into new biomass for all species analysed. Remediation performance attributed to abiotic processes such as precipitation and volatilisation were considered negligible through the attainment of average pH values (prior to pH adjustment) during the trials of 6.9 (±0.09) for *C.vulgaris*, 6.2 (±0.32) for *C.sorokiniana*, 7.7 (±0.30) for *M.aeruginosa*, 7.7 (±0.38) for *S.obliquus* and 7.7 (±0.15) for *Stigeoclonium* sp respectively. As such, when considering the pKa value for ammonium at 20^{o}C an estimated 2 - 4% (equivalent to 0.2 – 0.4 mg.L^{-1}) of removal can be contributed to volatised free ammonia at the peak pH value of 7.7 (±0.38); and minimal P precipitation through pH values lower than the required 8 – 9 required for precipitation with metal ions such as calcium (Ca) (Montastruc et al., 2003).

Species with a lower N composition were found to have an enhanced specific growth rate (Figure 3.1) through the reduced N requirements for growth. This is illustrated by a 1.5x increase in the final biomass concentration of 2.0 g(DW).L^{-1} compared to 1.3 g(DW).L^{-1} at the end of the trials by species with the lowest (0.06 mgN.mg(DW)$^{-1}$) compared to the highest (0.09 mgN.mg.(DW)$^{-1}$) N composition respectively. However, the increase in biomass concentration of those species with a lower N:P could not outcompete the remediation performance of those species with a greater N:P composition at a lower biomass volume. Overall, species with a greater N:P composition (high N and low P), where found to remediate ammonium and phosphate more efficiently than those species with a lower N:P under the specified conditions even when considering total biomass concentration.

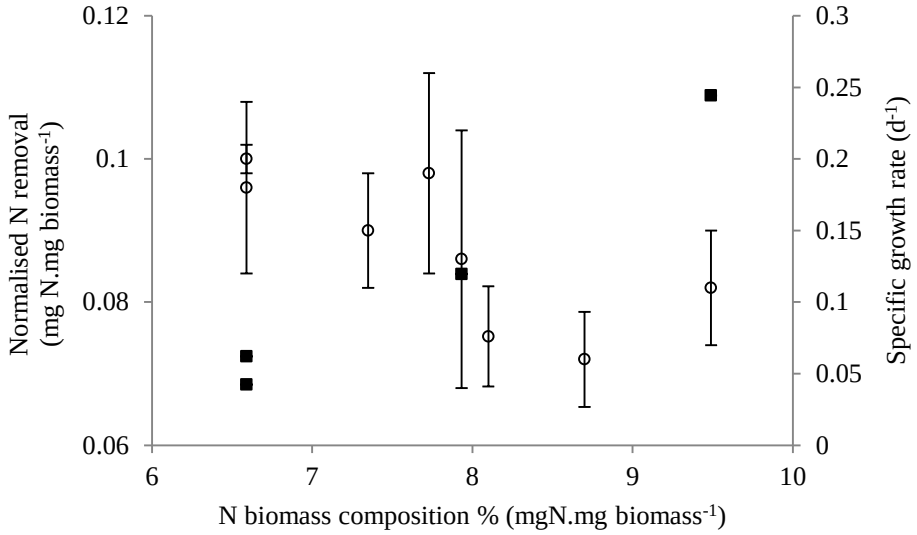

Figure 3.1 N remediation and specific growth in relation to species' internal N composition. N remediation (■) and specific growth rate (○). Uptake rates calculated using TSS data when available (mean ± standard error).

3.3.2 Wastewater nutrient remediation by *S.obliquus* and *C.vulgaris*

Two commonly used singled celled species initially characterised by a low and high N:P composition during cultivation; *S.obliquus* and *C.vulgaris*, were selected for trials with secondary effluent from a municipal wastewater treatment works. Ammonium concentration was fixed at 5 $mgN.L^{-1}$ and the phosphorus concentration varied between 0.5 and 10 $mgP.L^{-1}$, varying the medium N:P molar ratio between approximately 22.1 to 1.1 and encompassing a range of concentrations possible within secondary wastewater effluent (A. Brookes & P. Vale, 2011, pers. comms., 20 October). Increasing the P concentration and hence reducing the N:P ratio resulted in an increase in cell uptake for both species in terms of P (Figure 3.2a) and a decrease in cell uptake for N (Figure 3.2b) reflecting nutrient availability within the supplemented effluent.

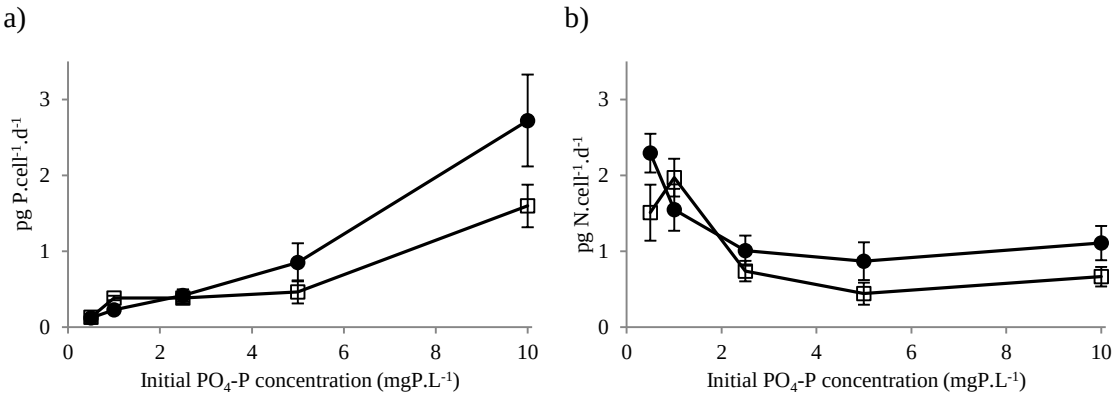

Figure 3.2 Cell uptake rate for a) PO_4-P and b) NH_4-N (mean ± standard error) by suspended *S.obliquus* (□) and *C.vulgaris* (●) in secondary wastewater effluent with varying initial PO_4-P concentration.

The phosphorus removal rate increased from 0.1 to 1.6 pgP·cell^{-1}·d^{-1} and 0.2 to 2.7 pgP.cell^{-1}.d^{-1} for *S.obliquus* and *C.vulgaris* respectively as the initial concentration increased from 0.5 – 10 mgP.L^{-1}. A minimum P remediation efficiency through the incorporation into new biomass of 47 – 82% and 18 – 77% for *S.obliquus* and *C.vulgaris* is estimated, prior to an increase in the effluent pH to values indicative of remediation through abiotic processes, with the initial biomass incorporation rates decreasing with increasing P concentration.

The removal of phosphate is associated with N removal through their respective roles in cellular metabolism (Loladze and Elser, 2011). In microalgae, N is mainly integrated into proteins that in turn links to the production of ribosomes and ribosomal RNA. Phosphate uptake is predominately associated with storage into the ribosomal RNA such that the observed function between P concentration and uptake rate requires sufficient N to ensure no restriction of protein synthesis. Previous work has shown that in low N environments, the uptake of P into the biomass remains low irrespective of the P concentration in the solution (Beuckels et al., 2015). In such cases the uptake rate also relates to the ability of microalgae to store available phosphate in time of surplus, through a luxurious uptake pathway where polyphosphate accumulates within the cells (Wang et al., 2010).

The pattern of remediation for NH_4-N were similar for both species and decreased as the concentration approached 2 mgP.L^{-1} prior to stabilising. Remediation rates of 0.7 – 1.5 pgN.cell^{-1}.d^{-1} and 1.1 – 2.3 pgN.cell^{-1}.d^{-1} for *S.obliquus* and *C.vulgaris* were demonstrated

(Figure 3.2b), with remediation through incorporation into new biomass estimated at a minimum of 54 – 99% and 38 – 93% for *S.obliquus* and *C.vulgaris* respectively, prior to an increase in the effluent pH and the contribution of volatilisation to total remediation.

Remediation performance was furthermore reflected in the cell uptake rate for both species remediating the equivalent of 18.5 – 82.1 and 36.3 – 95.6 fmolN.cell^{-1}.h^{-1} for *S.obliquus* and *C.vulgaris* respectively; greater than that reported for ammonia-oxidising bacteria (AOB) in wastewater of 0.03 – 53 fmolN.cell^{-1}.h^{-1} (Lydmark, 2006). When considering the difference in mass of AOB and microalgae, AOB concentrations of 10^{10} cells.gVSS^{-1} (Hallin et al., 2005) in comparison to approximately 5 x 10^{9} cells.g^{-1}.VSS for *Chlorella* (considering 7.7x10^{9} cells.gCOD^{-1} and 1.43 gCOD.gVSS^{-1} (Ras et al., 2011)) are reported. The associated mass uptake rates based on the higher ranges of 5.3x10^{-9} and 1.8x10^{-8} fmolN.gVSS^{-1}.h^{-1} for AOB and *Chlorella* respectively further demonstrate the effectiveness of microalgal cells for ammonium remediation.

Comparison of the two microalgal species in terms of cell uptake revealed similar levels of 0.4 (± 0.07) pgP.cell^{-1}.d^{-1} in low phosphate wastewater (< 2.5 mg.L^{-1}) (Figure 3.2a). In contrast, at higher initial phosphate concentrations of 5 mgP.L^{-1}, uptakes rates of 0.5 (±0.2) pgP.cell^{-1}.d^{-1} and 0.9 (± 0.3) pgP.cell^{-1}.d^{-1} were observed for *S.obliquus* and *C.vulgaris* respectively (Figure 3.2a). Nitrogen uptake was also slightly greater for *C.vulgaris* at higher P concentration consistent with *C.vulgaris'* higher N:P cell content but in contrast to previous work that showed that N concentration in biomass was independent of P supply (Beuckels et al., 2015) indicating other mechanisms. Notable differences were observed between species in terms of cell growth and associated alkalisation of the surrounding medium. The growth rate of *C.vulgaris* was lower and more consistent across all concentrations with a range of specific growth rates between 0.16 and 0.29 d^{-1} (in comparison to 0.51 and 0.71 d^{-1} for *S.obliquus*) suggesting the greater P and N uptake observed at higher P concentration was not due to cell growth. Luxury phosphate uptake has been demonstrated to take effect for *Scenedesmus* beyond a critical growth concentration of 1.5 mgP.L^{-1} (Azad and Borchardt, 1970). At concentrations beyond this level uptake through luxury consumption has been observed, with no impact on growth (Azad and Borchardt, 1970). Similar observations were found within this study, and supports the improved remediation performance at the higher concentrations

despite the narrow range of growth rates observed for the different PO_4-P concentrations. The lower growth rate observed for *C.vulgaris* resulted in a reduced degree of alkalinisation as evidenced by an average final pH of 10.9 for *S.obliquus* in comparison to 9.7 for *C.vulgaris*.

3.3.3 Discussion: implications for an immobilised microalgal reactor for tertiary wastewater nutrient remediation

The aim of the study was to examine the impact of variation in nutrient content of algal biomass on the associated nutrient uptake rates and understand the importance of algal species selection when operating a tertiary treatment system. Overall, the nutrient concentration in algal biomass is not fixed and so does not map to predictions based around the Redfield ratio (Hecky et al., 1993). Furthermore microalgae display flexibility in the nutrient concentrations in the biomass enabling adaptation to the local environment with nutrient uptake limited by the species' specific cellular quota for structural components and storage for future growth (Droop, 1968). Accordingly, design of microalgae reactors for wastewater treatment need to consider species selection and nutrient concentrations in the biomass and ability to adapt to external concentrations as it impacts on the maximum treatable loading rate and associated footprint.

Experimental results characterising nutrient uptake for the species *S.obliquus* and *C.vulgaris* were used to calculate cell concentrations required for 'optimal remediation' (<0.1 $mgP.L^{-1}$) from initial concentrations similar to those found within tertiary effluent. Required concentrations were predominately calculated through PO_4^{3-} remediation as phosphate is targeted for further reductions in consent through the WFD. Required cell concentrations for both *S.obliquus* and *C.vulgaris* were found to increase to > $3x10^6$ $cells.mL^{-1}$ for HRTs less than 24 h, with maximum cell concentrations of $8.5x10^7$ and $4.7x10^7$ $cells.mL^{-1}$ at a treatment time of 3 h for *S.obliquus* and *C.vulgaris* respectively for the higher P concentrations (Figure 3.3).

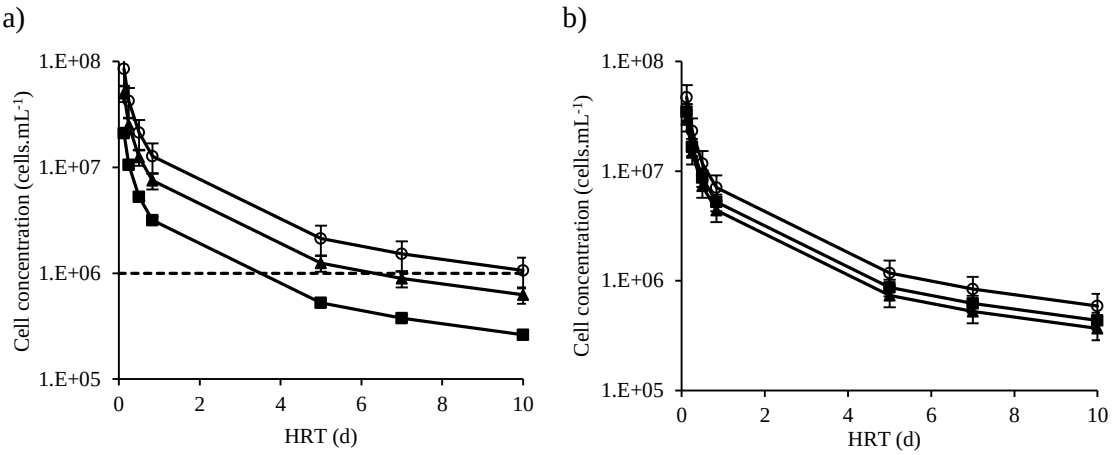

Figure 3.3 Optimal cell concentration for a) *S.obliquus* and b) *C.vulgaris* with HRT for influent PO_4-P concentrations of (■) 1, (○) 5 and (▲) 10 mgP.L^{-1} (mean ± standard error), (--) denotes approximate equivalent biomass concentration for a HRAP.

The required cell concentration for *C.vulgaris* was found to be less variable than *S.obliquus* with similar biomass concentrations necessary for the remediation of 1, 5 and 10 mg.L^{-1}. *C.vulgaris* is therefore considered a better option for the treatment of wastewater with a varying influent concentration. However, for both species, greater biomass concentrations were necessary for treatment at 5 mgP.L^{-1} than 10 mgP.L^{-1}due to a cell uptake rate three times greater when treating 10 mgP.L^{-1} highlighting the ability of microalgae to adjust their performance to suit a changing environment, with enhanced remediation through luxury uptake within nutrient rich environments (Eixler at el., 2006) thus the reduced biomass concentration at the higher P concentration.

Open pond systems (i.e. HRAPs) are reported to maintain an approximate biomass concentration of 0.2 g(DW).L^{-1}, equivalent to a cell concentration of up to 10^6 cells.mL^{-1} for *S.obliquus* (based on laboratory growth data). This concentration is sustained through the variation in light, temperature and biotic factors including zooplankton grazers and pathogens (Park et al., 2011). As such, HRTs >4 days are necessary to achieve an optimal level of treatment when using a HRAP through the biomass concentration achievable. To illustrate the consequence of this, a wastewater treatment works with population equivalence (PE) of 2,000 treating the standard 0.18 m^3.pe^{-1}.d^{-1} of effluent would require a HRAP with a surface footprint of 7,200 m^2 at a depth of 0.2 m and minimum HRT of 4 days. This footprint is considerably larger than that of conventional tertiary treatments such as rotating biological contactor (RBC) unit or trickling filter with land footprints of

40 – 50 m^2 (Butterworth et al., 2013). As such, a HRAP would be an unlikely solution for retrofitting to a site of 2,000 PE. To overcome limitations around treatable load and footprint an intensification of the algal biomass is required. Immobilisation enables biomass concentrations beyond 10^7 cells.mL^{-1} through either an increase in the cells per bead or the number of beads per unit volume, with example levels of up to 10^8 cells.mL^{-1} (Abdel Hameed, 2007) and biomass concentration of up to 3.3 g(DW).L^{-1} (Chevalier and De la Noue, 1985) reported, equivalent to the typical biomass concentration found within the activated sludge (AS) process (Metcalf & Eddy et al., 2003)

To illustrate the impact of immobilisation on intensification of microalgae based wastewater treatment, the cell concentration required for each influent concentration (Figure 3.3) was used to determine the required bead concentration (based on an initial internal bead concentration of 10^6 cells.$bead^{-1}$) (Figure 3.4). Calculations for PO_4-P removal considering 10^6 cells.$bead^{-1}$ found bead concentrations of 3.2 to 85.1 beads.mL^{-1} (1:12.5 to 2.1:1 bead:wastewater v/v) for *S.obliquus* and 4.4 to 47.1 beads.mL^{-1} (1:9 to 1.2:1) for *C.vulgaris* (Figure 3.4) for remediation of influent concentrations of 1, 5 and 10 mgP.L^{-1}.

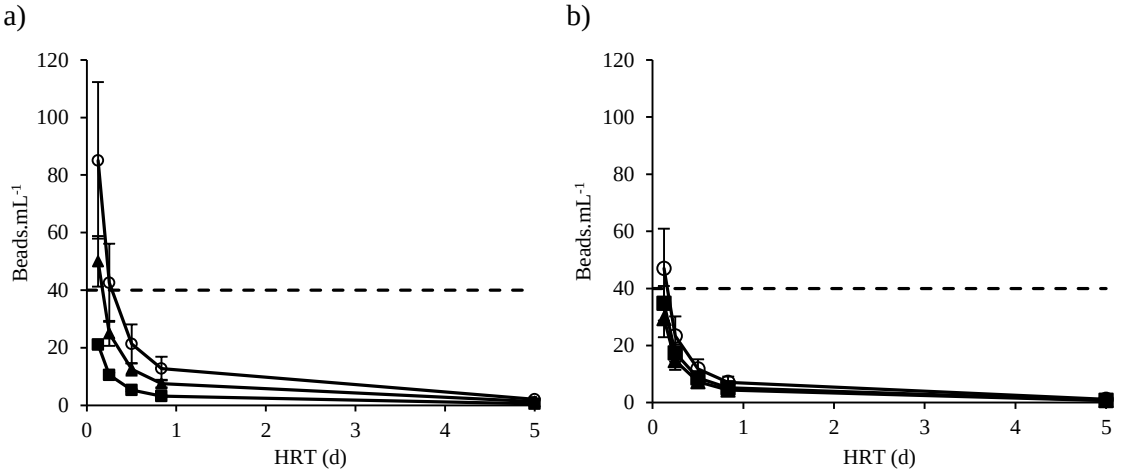

Figure 3.4 Corresponding bead.mL^{-1} concentration for a) *S.obliquus* and b) *C.vulgaris* with HRT up to 5 days for influent PO_4-P concentrations of (■) 1, (○) 5 and (▲) 10 mgP.L^{-1} (mean ± standard error), (--) denotes 1:1 (bead:wastewater v/v) and maximum bead·mL^{-1} concentration possible.

The required bead·mL^{-1} concentration was found to increase with the reduction in HRT due to the increased loading rate and required increase in biomass concentration. Overall,

a lower bead.mL^{-1} concentration was found for *S.obliquus* at the lower PO_4-P influent concentrations (1 mgP.L^{-1}) and *C.vulgaris* for the higher concentrations (> 5mgP.L^{-1}) (Figure 3.4) due to the increased cell uptake rate demonstrated by *C.vulgaris* during batch trial characterisation (0.13 – 1.6 and 0.12 – 2.7 pgP.cell^{-1}.d^{-1} for 0.5 – 10 mgP.L^{-1} for *S.obliquus* and *C.vulgaris* respectively) (Figure 3.2a) and the corresponding performance associated to the characterised internal N:P composition.

These calculated bead concentrations can be compared to observed experimental performance of an immobilised algae reactor of *S.obliquus* treating a PO_4-P concentration of 0.7 mg·L^{-1} at fixed bead concentration of 10 beads·mL^{-1} and variable HRTs of 3, 6, 12 and 20 h (Whitton et al., 2014). Similar residual concentrations, following the initial start-up period of 0.10, 0.17 and 0.11 mgP.L^{-1} were observed for 6, 12 and 20 h respectively confirming a suitable bead/biomass concentration. However, a reduction in residual performance at a 3 h HRT of 0.43 mgP.L^{-1} was observed suggesting the biomass concentration to be inadequate. Based on the calculated bead concentrations presented (Figure 3.4a), a concentration of approximately 13 beads·mL^{-1} (equivalent of an additional 10^6 cell.L^{-1} and approx. 0.2 g(DW).L^{-1}) would have provided the additional biomass necessary to remediate within the shortened retention time and as such, the predicted biomass concentrations presented in Figure 3.4 can be used to inform cell concentration through bead volume for *S.obliquus* and *C.vulgaris*.

Extending this to higher loading rates needs to consider other practical aspects which limit the applicable bead concentration to 1:1 v/v in order to minimise practical issues. These include sinking and crushing of beads under their own weight (Abdel Hameed, 2007) and self-shading restricting light penetration (Lau et al., 1995) which can contribute to a significant reduction in NH_4^+ remediation performance (Abdel Hameed, 2007) as well as improving irradiation efficiency.

When applying the maximum bead concentration (1:1 v/v) to the range of influent concentrations, a treatment period of 1.5 – 2.5 h for an effluent with a concentration < 1mgP·L^{-1} is achievable by immobilised *S.obliquus* and *C.vulgaris* (Figure 3.5). Treatment periods >3 h are then necessary for the remediation of effluents >2.5 mgP.L^{-1} by both *S.obliquus* and *C.vulgaris* with required HRTs of 6.3 h and 3.5 h for *S.obliquus* and *C.vulgaris* at 5mgP.L^{-1} respectively (Figure 3.5). In situations where immobilised algae

are used as a tertiary treatment the solution is unlikely to encounter influent concentrations greater than 5 $mgP.L^{-1}$. As such the required HRT is less than 3 h indicating the potential for effective use of microalgae without the need for large footprint technology.

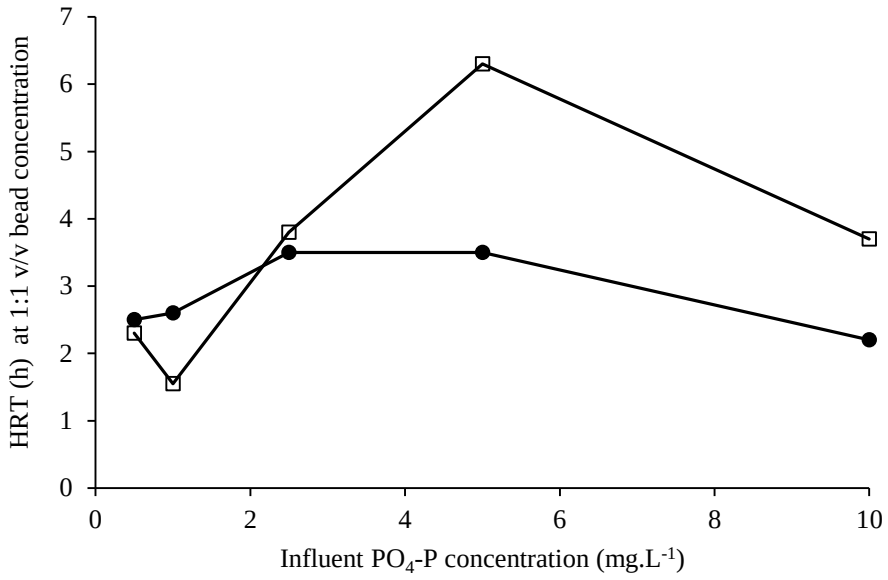

Figure 3.5 Theoretical minimum HRT at 1:1 (v/v) bead concentration for PO_4-P remediation by *S.obliquus* (□) and *C.vulgaris* (●).

Immobilisation also introduces an additional component in the form of the calcium-alginate beads that contain the microalgae and offers an additional uptake pathway**.** Adsorption trials with blank Ca-alginate beads found PO_4-P uptake by the resin material to be negligible across the tested PO_4-P concentrations (see Appendices, Figure B.1), confirming previous trials with blank alginate beads in sterile conditions (Cruz et al., 2013). However, within non-sterile wastewater Cruz et al. (2013) demonstrated a capacity of > 15 $\mu gP.g^{-1}$ over a 48 hour period with removal contributed to the formation of a concentrated biofilm layer supported by the bead's surface area and not directly through the adsorption capacity of the resin material. In contrast, uptake of NH_4-N resulted in removal efficiencies of 9.1, 20.6, 25.4 and 23.4% for NH_4-N at starting concentrations of 0.5, 2.5, 5 and 10 $mgN \cdot L^{-1}$ respectively, with an adsorption capacity of 6 $\mu gN \cdot g^{-1}$ determined through fitting the data to a Freundlich isotherm model (see Appendices, Figure B.2) and providing an additional pathway for nutrient removal when using immobilised systems. As such, this study provides a conservative estimate on the ability

of immobilised microalgae to remediate wastewater nutrient through species selection and biomass concentration as additional mechanisms, including the Ca-alginate resin and indirect methods of volatilisation and precipitation, would further enhance the overall remediation performance.

3.4 Conclusions

- A relationship between internal N:P composition and nutrient remediation is evident and can be considered when selecting a species for remediation. Species with a high N and low P internal composition remediate ammonium and phosphate more efficiently.
- Required biomass concentrations varied with wastewater characteristics and nutrient uptake abilities. When translated into immobilised beads, concentrations as low as 3.2 beads.mL^{-1} is possible for *S.obliquus* at HRT of 20 h.
- A HRT <3 h is impractical for an immobilised microalgal solution for concentration > 5 mgP·L^{-1}, due to the volume of beads required to achieve maximum remediation.

3.5 Acknowledgements

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Chapter 4. Tertiary Nutrient Removal from Wastewater by Immobilised Microalgae: Impact of N:P Ratio and Hydraulic Retention Time (HRT)

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Abstract

Immobilising microalgal cells within an alginate bead has been proposed as a process solution to overcome some of the barriers associated with the implementation of microalgae for wastewater remediation. Such barriers include the low algal biomass concentration in suspension coupled with the expense and challenge of harvesting the biomass after treatment. This work evaluated the performance and remediation mechanisms when using immobilised microalgae for continuous wastewater treatment under varying hydraulic retention times (HRT). Three domestic wastewaters with concentrations of orthophosphate (PO_4-P) ranging from 0.7 – 4.4 $mg.L^{-1}$, ammonium (NH_4-N) 0.3 – 4.2 $mg.L^{-1}$ and nitrate (NO_3-N) from 2.2 – 20.3 $mg.L^{-1}$, were treated with the freshwater species *Scenedesmus obliquus* immobilised within 2% caclium alginate. Trials were run in continuous operation at HRTs of 3, 6, 12 and 20 h. Removal rates for PO_4-P improved with increasing HRT, with average residual concentrations of 0.1 – 4.2 $mg.L^{-1}$ observed at 3 h HRT and 0.03 – 0.8 $mg.L^{-1}$ at 20 h HRT. Removal efficiency of 80% PO_4-P was observed at HRTs as low as 6 h. Ammonium remediation was not linked to HRT or NH_4^+ concentration, with > 70% removal observed for most wastewaters at all HRTs. Similar to phosphate, nitrate reduction improved with increasing HRT, with up to 85% removal at 20 h HRT. Remediation was achieved through a combination of mechanisms including uptake of nutrients by the immobilised biomass and an indirect route of volatilisation and precipitation as a by-product of photosynthesis and nutrient metabolism. As such, immobilised microalgae have been proven to be an effective

alternative solution for PO_4^{3-} and NH_4^+ remediation of wastewater effluents at HRTs of 20 h or less.

Keywords: algae, ammonium, hydraulic retention time, nitrate, nutrient removal, phosphate, wastewater

4.1 Introduction

Microalgae are photosynthetic organisms that assimilate mainly inorganic nitrogen (N) and phosphate (P) during their growth when present in wastewater effluent. As such, they have been proposed as an alternative solution to remediation of wastewater for both secondary and tertiary treatment (Lau et al., 1995; Martínez et al., 2000). For instance, the freshwater species *Scenedesmus obliquus* has been shown to achieve >98% remediation for total phosphate (TP) and ammonium (NH_4^+). Both contaminants were reduced to <0.3 $mg.L^{-1}$ over a 7 day period when treating a domestic secondary wastewater with an influent concentration of 21.3 $mg.L^{-1}$ NH_4-N and 3.9 $mg.L^{-1}$ PO_4-P (Martinez et al., 2000). The application of algal based technologies for nutrient removal offers a number of advantages over traditional nutrient removal options. This is especially the case for wastewater treatment works (WWTWs) that are trying to achieve final effluent concentrations of less than 0.5 $mg.L^{-1}$ PO_4-P, in line with the requirements of the water framework directive (WFD). When using algal systems, the residual biomass has value as a bioenergy source following appropriate pre-treatment (Ometto et al., 2014b) and hence offers the possibility of meeting low discharge consents in an energy neutral, sustainable manner. Further, nutrients can also be recovered from the algal biomass. Another advantage of algal treatment systems is the perception that they are more natural which helps to promote the public's receptivity of such processes.

To date, the majority of research conducted on wastewater nutrient removal with microalgae has focused on the use of high rate algal ponds (HRAP) (Christenson and Sims, 2011) containing a suspended culture of microalgae and bacteria at biomass concentrations of up to 0.2 $g(DW).L^{-1}$ (Craggs et al., 2012). Typical hydraulic retention times (HRT) from 4 – 10 days are required resulting in large footprints (Picot et al., 1992). Other studies have used much higher biomass concentrations in suspended reactors for very effective treatment of specific types of effluent that had much higher N and P concentrations than typical municipal wastewater. For example, treatment of

concentrated urine was achieved in a reactor with algal biomass concentrations over 8 g. L^{-1} (Tuantet et al., 2014). Similarly, an industrial wastewater from a pharmaceutical/biotechnology facility treated water with an algal biomass of >4 $g.L^{-1}$ (Van Wagenen et al., 2015). However, in all suspended systems, algae then need to be removed (harvested) prior to discharge, requiring large coagulant demands or energy inputs (Ometto et al., 2014a). If not removed, the algae will contaminate the receiving water body where they are reported to increase suspended solids and contribute 60 – 90% of the treated effluent biological oxygen demand (BOD) (Schumacher and Sekoulov, 2002). Such attributes restrict the attractiveness of suspended microalgal technologies, particularly in locations with limited land availability and concerns over energy and chemical use.

The immobilisation of microalgae by confining the living microalgal cells within a gel that is shaped into beads overcomes many of these barriers (Mallick, 2002). These gels are hydrophilic in nature, with small pores to enable the diffusion of wastewater to the entrapped microalgal cells (de-Bashan and Bashan, 2010). Immobilisation enables hyper concentration of biomass to over 3 $g(DW).L^{-1}$ in 2 – 3 mm diameter beads (Chevalier and De la Noue, 1985). The ability to increase the biomass concentration by a factor of up to 15 over most HRAP suspended systems through immobilisation enables a greatly reduced reactor footprint. Furthermore, immobilisation provides a significant advantage when the biomass is harvested, as the algal beads can be easily settled from the water by gravity without the addition of coagulant chemicals. This also prevents biomass from washing out of the reactor (Travieso et al., 1992; Mallick and Rai, 1994). It is, however, acknowledged that the immobilisation process itself requires resources that may negate some of the perceived benefits associated with the easy separation of immobilised algae.

The biomass intensification afforded by immobilisation has been found to greatly enhance the nutrient remediation potential of microalgae compared to suspended growth systems (Mallick and Rai, 1994). For instance, the contact time required to achieve 100% NH_4-N and 95% PO_4-P remediation was observed to be within 24 h for an immobilised system compared to days for HRAP (García et al., 2000). The ability of immobilised microalgae to remediate synthetic wastewater in batch or semi-continuous treatment is well documented (Mallick and Rai, 1994; Tam and Wong, 2000; Jiménez-Pérez et al.,

2004). However, there are limited studies on remediation performance within real wastewater effluents (Ruiz-Marin et al., 2010) and continuous operation simulating real world conditions. One such study by Travieso et al. (1992) analysed the remediation performance of immobilised microalgae in the treatment of primary effluent using two reactor configurations: a packed bed with an 8 h HRT and a fluidised bed with an 18 h HRT. The packed bed achieved a removal efficiency of approximately 67% PO_4-P and 76% NH_4-N in comparison to 72% PO_4-P and 82% NH_4-N for the fluidised bed. The improved performance by the fluidised bed was attributed to the increased contact between the effluent and immobilised beads (Travieso et al., 1992; Travieso et al., 1996). In comparison, Filippino et al. (2015) found a continuous system with a 6.5 h contact time to remediate > 80% total nitrogen (TN) and between <10 – 100% PO_4-P under differing lighting conditions and CO_2 addition.

The current paper extends previous work to specifically investigate the role of HRT using the same reactor configuration on the remediation performance when using immobilised algae for tertiary treatment of real wastewaters under continuous operation. Three different wastewater sources have been used to cover the typical range of N:P ratios that are commonly encountered for tertiary treatment to provide an understanding of performance and the mechanism of remediation.

4.2 Materials and Methods

4.2.1 Wastewater

Secondary wastewater effluent was collected from three wastewater treatment sites located in the Midlands and South East of the UK. Sites A, B and C represent wastewater treatment works with a population equivalent (PE) of 200,000, 3,000 and 32,000 respectively, with effluent collected following secondary treatment by trickling filters (site A and B) and an oxidation ditch (site C). Average wastewater characteristics during the experimental period are summarised in Table 4.1. Effluent from site C was supplemented with NH_4Cl and KH_2PO_4 to increase residual concentrations and compensate for seasonal upstream dosing. Wastewater was used upon collection, with the remainder stored at 4^oC during the period of the trial until use.

Table 4.1 Average wastewater characteristics over experimental period.

Parameter ($mg.L^{-1}$)	Site A	Site B	Site C[a]
PO_4-P	0.7	4.4	1.1
NH_4-N	4.2	0.3	2.6
NO_3-N	20.3	29.1	2.2
N:P (molar)	78	15	10
pH	7.8	7.3	7.7

[a]Average concentrations of 0.3 $mg.L^{-1}$ PO_4-P and 0.1 $mg.L^{-1}$ NH_4-N prior to supplementation.

4.2.2 Microalgae cultivation and immobilisation procedure

The freshwater species *Scenedesmus obliquus* (276/3A) was obtained from the Culture Collection for Algae and Protozoa (CCAP) (Oban, UK) and cultured in 50 L of Jaworski medium (SI, Appendix B.1). This medium was chosen as a result of the enhanced algal growth observed during preliminary experiments. Algae were cultured in a temperature controlled room at 20^{o}C under constant mixing by a circulation pump (900 $L.h^{-1}$) (Hydor Koralia Nano 900). Cultures were illuminated at a light intensity of 100 – 150 $\mu mol.m^{-2}.s^{-1}$ at the culture surface, under constant light to adapt the biomass for continual activity and the accompanying remediation necessary for continuous wastewater treatment. Microalgal biomass was harvested prior to the onset of stationary growth phase to enable maximum biomass recovery. The biomass was stored overnight at 4^{o}C prior to immobilisation.

Beads were prepared following the method of Tam and Wong (2000) and Ruiz-Marin et al. (2010) to achieve a final 2% sodium alginate (Na-alg) concentration followed by solidification within 2% $CaCl_2$. Algal biomass was mixed within the Na-alg gel then passed through a peristaltic pump and dripped into a magnetically stirred $CaCl_2$ solution from a height of 30 cm. Approximately 4,000 beads per 100 mL gel were formed with an approximate biomass concentration of 10^5 $cells.bead^{-1}$. Beads with an average diameter of 3 mm were left to solidify within the $CaCl_2$ solution overnight and stored in the dark at 4^{o}C for a period of 24 – 48 h prior to use. Preliminary experiments showed that this had no effect on cell viability once algal beads were placed in the light again. Beads were rinsed several times with DI water to remove any surplus $CaCl_2$. No cell lysis was

observed during this procedure. The beads themselves played no significant role in nutrient removal as confirmed by a negligible change in N and P from batch adsorption tests when beads with no algae were added to wastewater.

The cell.bead^{-1} concentration was confirmed by removing a sample of 10 beads and dissolving them in a known volume of 2% sodium citrate. The cell concentration was recorded in triplicate using a haemocytometer and light microscope (Olympus, BH Series) and back-calculated to confirm approximately 10^5 cells.bead^{-1}.

4.2.3 Experimental setup for continuous treatment

Trials were run in continuous operation within an Algem™ Labscale Photobioreactor (Stewartby, UK) (Figure 4.1) at 20^{o}C under constant light at a photon irradiance of 200 μmol.m^{-2}.s^{-1} provided to the base of the photobioreactor over a surface area of 133 cm^2. An intensity of 200 μmol.m^{-2}.s^{-1} was selected following preliminary experiments carried out to observe growth and nutrient remediation performance of immobilised *S. obliquus* under varying light intensities.

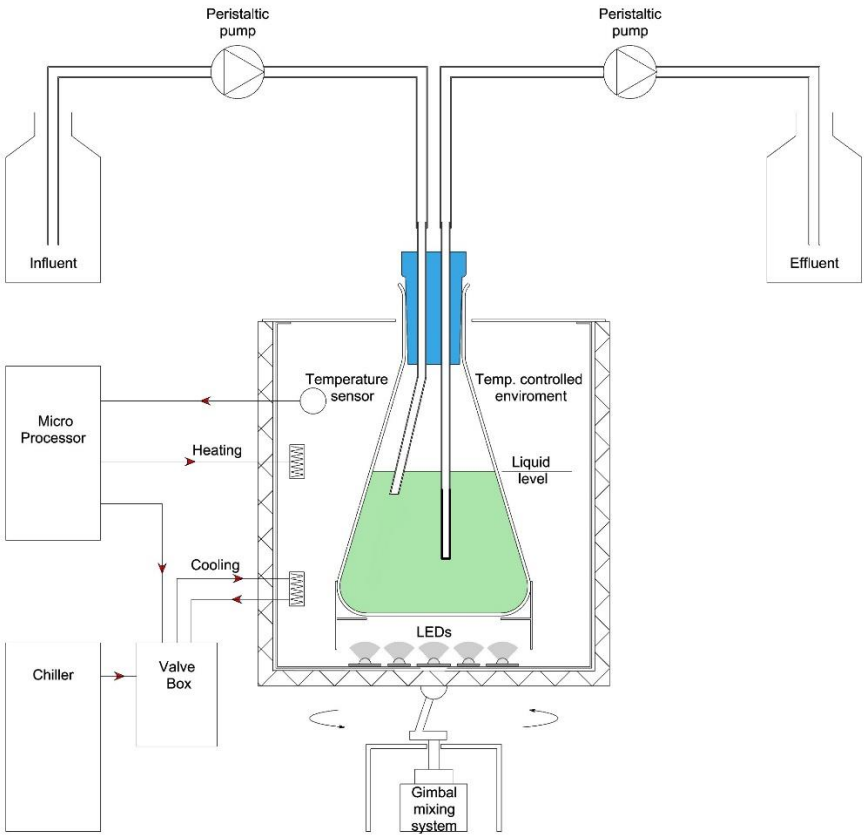

Figure 4.1 Schematic of the Algem™ Labscale Photobioreactor and auxiliary equipment.

Continuous flows were achieved using a peristaltic pump simulating HRTs of 3, 6, 12 and 20 h. Influent was fed to the top of the reactor and extracted from the base of the reactor below the bed of beads. Reactors were mixed via a gimbal system (Figure 4.1) at 120 rpm with fluidisation of the bed limited to the lower third of the vessel. The HRT here is defined as the retention time of the effluent within the reactor, and not solely the time spent within the bed of bead. Retention time within the bed of beads was significantly less than the reactor HRT (approximately one third of the quoted HRT) but was not directly measured, so total HRT is used throughout.

The 1 L reactor conical flasks in the photobioreactor (Figure 4.1) were filled with 600 mL of wastewater effluent with a bead concentration of 10 beads.mL^{-1}, as suggested by Abdel Hameed (2007), with an approximate initial dry weight concentration of up to 1 g.L^{-1} of solely algal biomass. The beads were retained within the reactor throughout the period of the trials. Trials were terminated when the residual concentration of the target nutrients returned to that of the feed (defined as performance breakthrough) or when a substantial

release of microalgal cells were observed within the reactor following bead deterioration, monitored through measurement of bead diameter using an electronic calliper.

4.2.4 Sample analysis and biomass growth

Samples of the treated effluent (excluding the microalgal beads) were taken twice daily during the start-up period and once a day when performance stabilised. Daily analysis included pH, NH_4-N, NO_3-N and PO_4-P. Analysis of the total and dissolved fraction of phosphorus were completed. Insoluble phosphorus was determined by subtracting the dissolved fraction from the total concentration. Dissolved PO_4-P was analysed daily following syringe filtration at 0.45µm (Millipore, DE), whereas total phosphate (TP) was analysed every 2 – 3 days for unfiltered samples. Remediation performance was quantified as the difference in the influent and effluent concentration of the continuous flow, with removal associated to direct uptake and precipitation. Residual concentrations were analysed in duplicate using Spectroquant test kits (Merck Millipore) and read via a Spectroquant Nova 60 spectrophotometer and reported as the mean ± standard error when possible. Elemental analysis of precipitation was investigated through a scanning electron microscope attached with an energy dispersive electron probe X-ray analyser (SEM–EDS, FEI XL30).

Growth of the immobilised biomass was analysed by dissolving a sample of 10 beads from the reactors throughout the experimental period as previously described. The specific growth rate was then calculated using Equation 4-1, where µ = specific growth rate (d^{-1}), x1 and x2 the number of cells.$bead^{-1}$ at time t1 and t2 respectively.

$$\mu = \frac{\ln(\frac{x1}{x2})}{t2 - t1} \qquad \text{Equation 4-1}$$

Suspended biomass released by the beads in to the reactor were found to contribute to approximately 0.4% of the total biomass concentration from preliminary 24 h batch trials. The contribution of the suspended biomass was therefore considered negligible in this work through the selection of HRTs ≤ 20 h for the continuous trials. Contribution of bacteria to removal of contaminants was also considered negligible as the reactors were not seeded with bacterial biomass and the treated effluent was not recycled.

4.3 Results and Discussion

4.3.1 Phosphate remediation

A consistent treatment profile was observed across each of the wastewater samples analysed from all sites and HRTs with an initial period where the effluent PO_4-P decreased before reaching a plateau. For example, site A samples demonstrated more than 80% removal at HRTs of 3, 6, 12 and 20 h over a period of approximately 2, 1, 3 and 5 days (Figure 4.2). Treatment efficiencies were similar to those observed by Filipinno et al. (2015) for continuous treatment of wastewater at an average influent concentration of 0.2 – 0.5 $mg.L^{-1}$ PO_4^{3-} (similar to the characteristics of Site A) using immobilised *Chlorella vulgaris* at a contact time of 6.5 h. The present study demonstrated a similar level of performance at a further reduced HRT of ≤ 6 h (equivalent to a ≤ 2 h bead contact time).

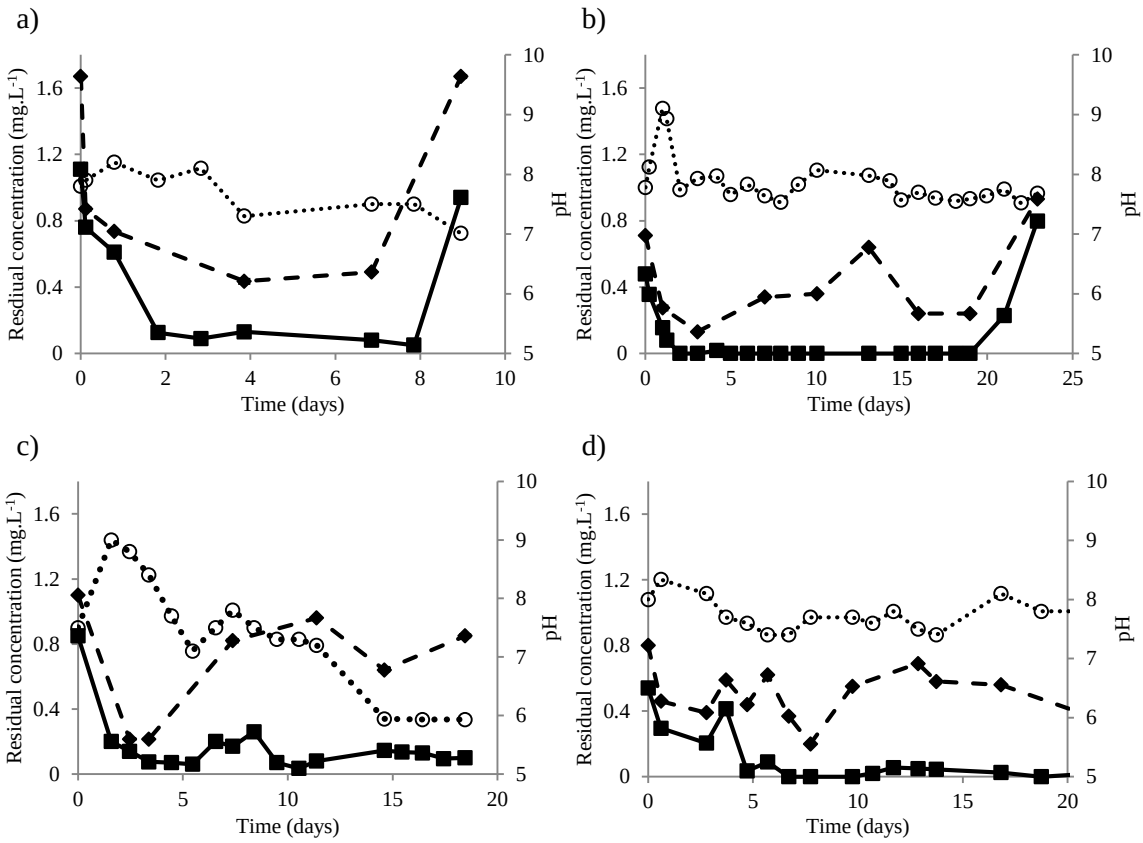

Figure 4.2 Phosphate remediation for site A for a) 3 h, b) 6 h, c) 12 h and d) 20 h HRT. PO_4-P (■), TP (♦) and pH (○). Average influent concentration 0.70 $mg.L^{-1}$ PO_4-P and 1.1 $mg.L^{-1}$ TP.

The residual concentration of PO_4-P averaged 0.10, 0.001, 0.12 and 0.03 $mg.L^{-1}$ until day 8, 19, 19 and 19 when a breakthrough in concentration (HRTs 3 and 6 h) or deterioration of the beads (HRTs 12 and 20 h) was observed. Comparison of samples across the sites revealed that following the initial start-up period, but prior to breakthrough, the plateaued PO_4-P residual concentration for site A and C samples achieved < 0.12 $mg.L^{-1}$ for all HRTs (excluding 3h for the site C sample) (Table 4.2). The similar levels of performance observed between site A and C samples were due to the similar PO_4-P influent concentration (as discussed in Section 2.3.1). The samples from site B were not reduced to such low levels, achieving the lowest residual concentration of 0.77 $mg.L^{-1}$ at 20 h HRT. The reduced performance was attributed to the higher PO_4-P influent concentration which was up to 6 and 4 times greater than site A and site C samples respectively, suggesting load limiting conditions had been reached.

Table 4.2 PO_4-P residual concentration following initial start-up period and prior to breakthrough, peak pH and maximum removal contribution through precipitation.

HRT (h)	PO_4-P residual		Peak pH	Max. precipitation contribution (%)
	$mg.L^{-1}$	Std error		
Site A				
3	0.10	0.01	8.2	43.7
6	0.001	0.001	9.1	50.7
12	0.12	0.02	9.0	80.5
20	0.03	0.01	8.3	80.0
Site B				
3	4.17	0.59	7.3	nd
6	2.21	0.58	8.2	33.3
12	0.88	0.06	10.6	81.7
20	0.77	0.23	11.0	49.1
Site C				
3	0.41	0.10	8.6	nd
6	0.07	0.02	9.7	80.3
12	0.04	0.01	10.4	88.5
20	0.08	0.03	11.1	44.0

nd = not determined.

The site effluents were characterised by a molar N:P ratio of 78, 15 and 10 for site A, B and C respectively. These ratios differ to the recommended N:P of 16 for marine microalgae (Redfield, 1934), but are more suited to the range of N:P ratios deemed suitable for freshwater species of 8 – 45 (Hecky et al., 1993). The growth rate of the immobilised biomass (for trials terminated through performance breakthrough) ranged between 0.22 – 0.42 d^{-1} with an increase in cell concentration from 10^5 to approximately 10^6 cells.$bead^{-1}$ suggesting phosphorus was not limiting even at the higher ratio. These findings are supported by previous work demonstrating effective N and P removal by immobilised microalgae within growth mediums characterised by N:P ratios between 4 – 230 (Filippino et al., 2015).

A change in the removal pathway was observed during the operating cycle for all HRTs and site samples. To illustrate for the site A sample, the TP residual concentration initially followed the remediation profile for PO_4-P (Figure 4.2) suggesting PO_4-P to be the predominant form of phosphorus within the effluent. Microalgal remediation of PO_4-P is predominantly through a biological uptake pathway (Larsdotter, 2006) and as such remediation during the initial stages of the cycles were attributed to microalgal assimilation. However, as the operating cycle progressed, the residual TP concentration began to change with an increase in concentration observed on days 4, 7, 7 and 10 for 3, 6, 12 and 20 h HRT runs respectively (for site A sample). The concentration remained higher than the PO_4-P residual and eventually returned to that of the feed when breakthrough was observed (Figure 4.2a and b) or the trial was stopped due to bead deterioration (Figure 4.2c and d). In addition, the effluent pH increased from an average of 7.7 to 8.2, 9.1, 9.0 and 8.3 for HRT of 3, 6, 12 and 20 h respectively (Figure 4.2, Table 4.2). The pH increase modifies the physiochemical environment (Nurdogan and Oswald, 1995) causing the onset of phosphate precipitation with calcium (Ca) and magnesium (Mg) cations found within the wastewater (Larsdotter et al., 2007). Precipitation then proceeds, even when the pH reduces to neutral, and continues to contribute to remediation (House, 1999).

The change observed in TP:PO_4-P during the cycle is congruent with a switch in the removal pathway from microalgae uptake in the initial stages to chemical precipitation during the remainder of the cycle. The precipitation pathway is driven by the increase in

pH as a consequence of photosynthesis and its commensurate consumption of the inorganic carbon source, i.e. the bicarbonate ion (HCO_3^-). This produces hydroxyl ions (OH^-), creating a localised increase in pH (Nurdogan and Oswald, 1995; Larsdotter et al., 2007; Kim et al., 2010). Analysis of the precipitate revealed it to be predominately amorphous calcium phosphate.

Alkalisation of the wastewater followed by an increase in residual TP concentration was demonstrated in all wastewaters at all HRTs, with a maximum pH of 11.1 after 2 days observed for the 20 h HRT trial for the site C sample (Table 4.2). Across all the trials, precipitation accounted for a maximum of 33.3 – 88.5% for phosphorus removal (Table 4.2) with greater contribution through precipitation generally occurring at the longer HRTs. However, the percentage contribution by precipitation at 20 h HRT reduced to between 44 – 49 % even though the pH peaked at 11 for samples from site B and C. Such observations are congruent with a switch in the relative competition for Ca between carbonate and phosphate. While the formation of calcium phosphate remains thermodynamically favourable, the relative precipitation kinetics significantly favour calcium carbonate formation reducing the amount of phosphate precipitate (Montastruc et al., 2003).

Equivalent findings have been reported in suspended systems with, for instance, precipitation accounting for 16 – 63% P removal for a suspended culture of *Monoraphidium* species at a HRT of 5 days growing within sterile filtered domestic wastewater (Larsdotter et al., 2007). This extends previous discussions concerning the significant role of indirect remediation (Mesplé et al., 1996; Garcia et al., 2000) to include systems based on immobilised microalgae. The increased contribution in the immobilised systems compared to suspended systems is attributed to the greater algal biomass concentrations involved; with suspended systems up to an order of magnitude less concentrated than immobilised systems.

4.3.2 Nitrogen remediation and pH

4.3.2.1 Ammonium remediation

Remediation of NH_4-N exhibited a similar pattern of remediation to PO_4-P with a period of initial decrease to a plateau which was consistent for all site samples and HRTs. To

illustrate, the site A sample achieved greater than 70% removal following an initial start-up period of 1, 3 and 5 days for 6, 12 and 20 h HRT respectively (equivalent to approximately 2, 4 and 6.7 h bead contact time) with corresponding averaged residual concentrations of 0.06, 0.25 and 0.35 mg.L^{-1} (Figure 4.3). This pattern of performance was not seen for the 3 h HRT trial (~1 h bead contact time), with a 14.3% reduction after 4 days prior to an increase in NH_4-N residual which returned to that of the feed by day 7 (Figure 4.3). The enhanced uptake and improved residual concentrations for NH_4-N in comparison to PO_4-P during these trials is congruent with the greater nitrogen content of algal biomass (around 10 times that of phosphorus) leading to the enhanced assimilation of nitrogen in comparison to phosphorus (Nurdogan and Oswald, 1995)

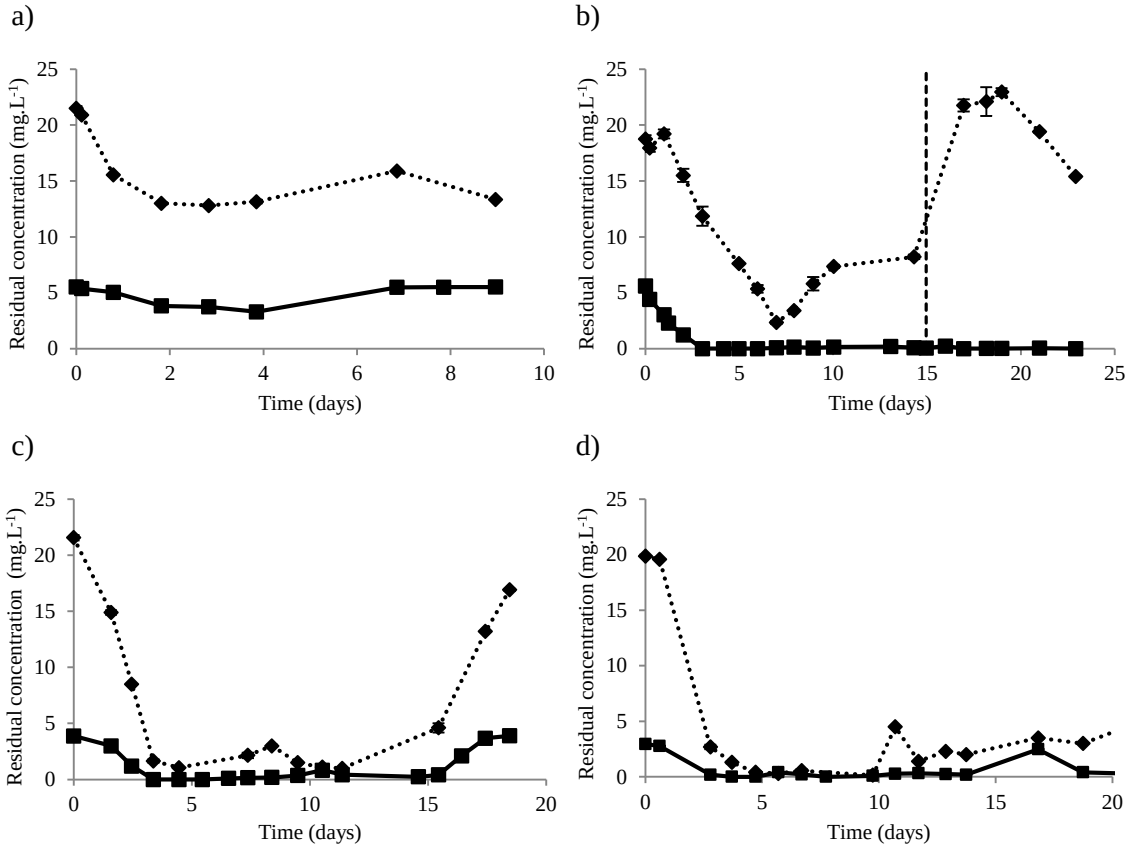

Figure 4.3 Ammonium and nitrate remediation for site A samples for a) 3 h, b) 6 h (addition of fresh feed on day 15.0), c) 12 h and d) 20 h HRT. NH_4-N (■) and NO_3-N (♦). Average influent concentration 4.2 mg.L^{-1} NH_4-N and 20.3 mg.L^{-1} NO_3-N.

The residual NH_4-N concentration, following the start-up period and prior to breakthrough, did not show considerable improvement with increasing HRT (Table 4.3). Furthermore, there were no substantial differences in the residual NH_4-N concentration

despite differences in influent concentration (excluding site A sample and 3 h HRT). These results were comparable to > 90% TN remediation efficiency observed by Filippino et al. (2015) for immobilised *C.vulgaris* at HRTs of 6.5 and 20 h and consistent with the system remaining non-limiting up to loading rates of at least 20.8 $g.m^{-3}.d^{-1}$.

Table 4.3 NH_4-N residual concentration following initial start-up period and prior to breakthrough and cumulative NO_3-N residual average.

HRT (h)	NH_4-N		NO_3-N	
	$mg.L^{-1}$	Std error	$mg.L^{-1}$	Std error
Site A				
3	3.63	0.16	14.1	0.9
6	0.06	0.02	9.8	1.7
12	0.25	0.08	2.4	2.0
20	0.35	0.07	3.0	1.6
Site B				
3	0.17	0.09	14.6	10.8
6	0.02	0.02	4.7	3.7
12	0.03	0.01	7.1	2.1
20	0.22	0.10	3.0	2.5
Site C				
3	0.003	0.002	0.3	nd
6	0.02	0.01	0.1	nd
12	0.03	0.004	nd	nd
20	0.03	0.01	0.3	0.3

nd = not determined.

4.3.2.2 Nitrate remediation and pH

The remediation of nitrate (NO_3-N) was observed following the uptake of NH_4-N showing the potential of the immobilised process to provide TN remediation (Figure 4.3). Reduction of NO_3-N for the complete cycle for the site A sample of 31%, 52%, 88% and 85% was observed for 3, 6, 12 and 20 h HRT respectively. The residual concentration was maintained throughout the trial and the system only showed a deterioration in performance following the addition of fresh feed for the 6 h HRT and bead deterioration after 19 days for 12 h HRT (Figure 4.3). Comparable NO_3-N remediation was observed for samples from site B due to the similar NO_3-N influent concentration (Table 4.3).

Samples from site C in contrast, represents a wastewater with a greatly reduced average NO_3-N concentration of 2.2 mg.L^{-1} and, as such, effective remediation to < 0.5 mg.L^{-1} was observed over the course of the trials (Table 4.3).

The impact of the relative removal of both nitrogen species was observed in relation to the evolution of pH (Figure 4.4). The sample from site A, with an average influent concentration of 5.6 mg.L^{-1} NH_4-N and 18.0 mg.L^{-1} NO_3-N peaked at a maximum pH of 9.1 before reducing to an average of 7.9 for the remainder of the run for the 6 h HRT (Figure 4.4a). The sample from site B, with a lower average NH_4-N influent concentration of 1.6 mg.L^{-1} and a higher NO_3-N concentration of 27.5 mg.L^{-1}, peaked at a maximum pH of 10.6 before averaging 9.2 for the remainder of the trial for the 12 h HRT (Figure 4.4b). The pH of the effluent mirrored that of the NO_3-N residual concentration for site B (Figure 4b), with a reduction from a pH of 10.0 to 7.2 after 19 days following an increase in NO_3-N residual from 2.4 to 20.1 mg.L^{-1}. The ratio of NH_4-N to NO_3-N influences pH as assimilation of NO_3^- results in a net H^+ uptake by co-transportation through the ATPase extrusion pump within microalgal cells whereas assimilation of NH_4^+ creates a net release of H^+ (Ullrich, 1983). Furthermore, whilst not directly measured, the estimated volatilisation of free ammonia (from its pKa) was between 1 – 98 % at the peak pH values of 7.3 – 11.1 (Table 4.2). The elimination of NH_3 by volatilisation at the higher pH values further facilitates alkalisation of the localised medium through the NO_3^- uptake mechanism.

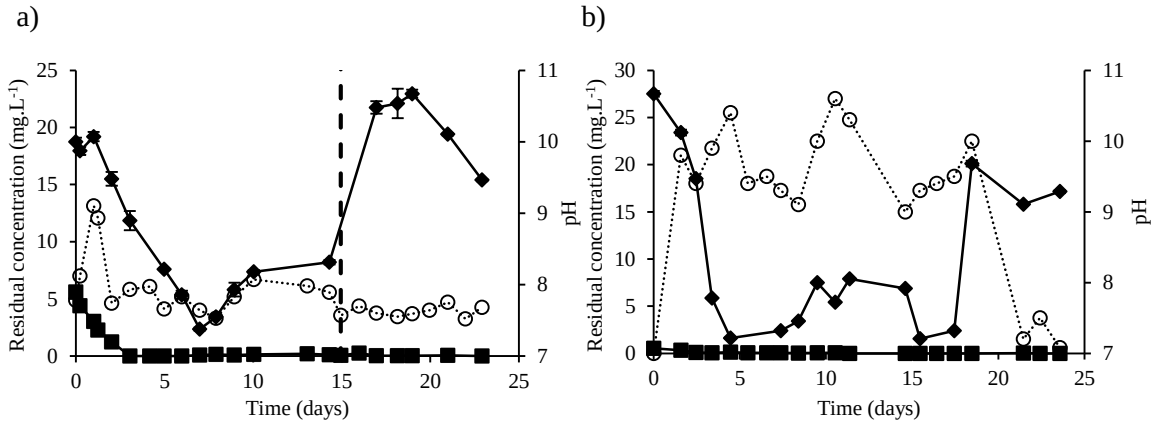

Figure 4.4 Ammonium and nitrate residual concentration and pH for a) site A sample at 6 h HRT, and b) site B sample at 12 h HRT. Fresh feed introduced on day 15.0 for site A. NH_4-N (■), NO_3-N (♦) and pH (○).

4.3.3 Bead remediation characteristics

The nutrient removal rate ranged from 0.03 to 74.6 mgP.h^{-1}.10^{-6} beads (equivalent to 0.003 to 0.75 µgP.h^{-1}.10^{-6} cells) (Figure 4.5a) and 0.03 to 142.7 mgN.h^{-1}.10^{-6} beads (equivalent to 0.003 to 1.43 µgN.h^{-1}.10^{-6} cells) (Figure 4.5b) with an average of 0.16 µgP.h^{-1}.10^{-6} cells and 0.43 µgN.h^{-1}.10^{-6} cells when considering only those trials which were halted through performance breakthrough. Such data demonstrate enhanced cell uptake rates when compared to previous studies that have used immobilised algae treating urban wastewater. For example, for a feed concentration of 32.5 mg.L^{-1} NH_4-N and 2.5 mg.L^{-1} PO_4-P operating in 50 h batch trials, uptake rates of 0.04 µgP.h^{-1}.10^{-6} cells and 0.62 µgN.h^{-1}.10^{-6} cells were observed (Ruiz-Marin et al., 2010). In comparison to a suspended culture under the same conditions, a similar PO_4-P uptake rate of 0.05 µgP.h^{-1}.10^{-6} has been shown, in contrast to a reduced NH_4-N remediation of 0.18 µgN.h^{-1}.10^{-6} cells (Ruiz-Marin et al., 2010).

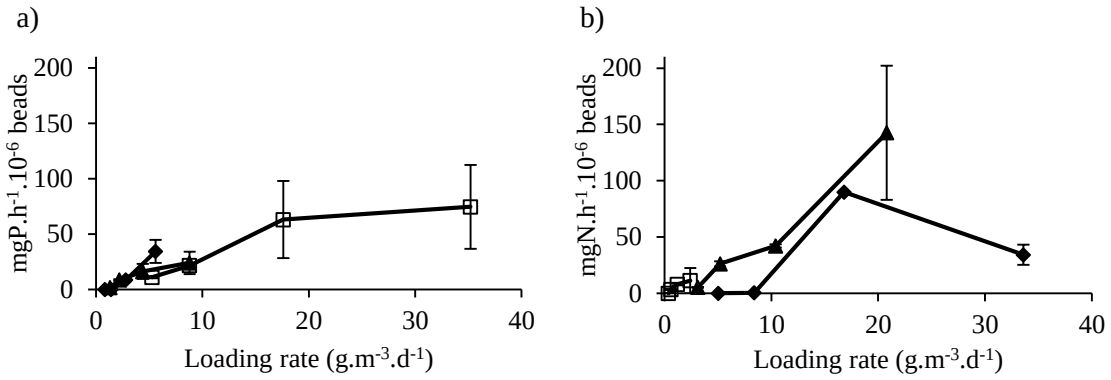

Figure 4.5 Bead removal rate for a) PO_4-P and b) NH_4-N for site A (♦), site B (□) and site C (▲).

The bead PO_4-P uptake rate increased until a loading of approximately 20 g.m^{-3}.d^{-1} after which the rate levelled out (Figure 4.5a) with results suggesting a maximum uptake rate of approximately 75 mgP.h^{-1}.10^{-6} beads. A similar relationship was observed for NH_4-N (Figure 4.5b); however, a rapid decrease was observed at 33.6 g.m^{-3}.d^{-1} concurrent with performance breakthrough within 8 days for the site A sample at 3 h HRT. Accordingly, the data suggests a maximum effective loading rate between 20.8 g.m^{-3}.d^{-1} and 33.6 g.m^{-3}.d^{-1}, corresponding to a maximum uptake rate of approximately 116 mgN.h^{-1}.10^{-6} beads.

The effective cycle time was defined by either performance deterioration or loss of bead integrity. In cases of lower nutrient loading, the cycle time was defined by performance

breakthrough. This occurred at all HRTs for site C samples and at HRTs of 3 and 6 h for site A samples and 12 and 20 h for site B samples with a reduction in cycle run time from 24 days to 3 days as the beads treated 0.1 to 0.8 µgP.bead^{-1}d^{-1} (Figure 4.6a). In such cases, an increase in residual effluent concentration was observed over a period of 2 – 5 days, providing a signal that the operating cycle had ended and represents a convenient method for cycle time control. No such relationship between specific growth rate or the final bead biomass concentration and the cycle run time was evident.

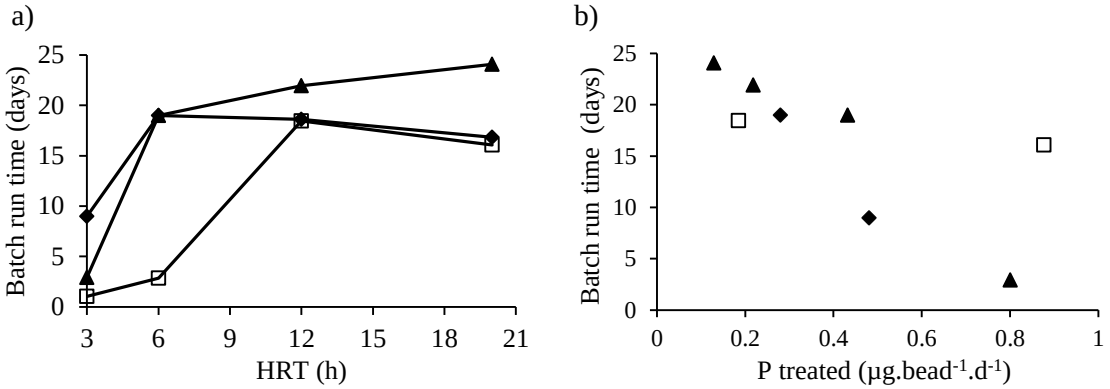

Figure 4.6 a) Batch run time for PO_4-P remediation for all HRTs and b) amount of P treated and run time (excluding trials with bead deterioration) for site A (♦), site B (□) and site C (▲).

Site A samples at 3 and 6 h HRT and site B samples at 12 and 20 h HRT demonstrated bead degradation and continued to remediate the influent wastewater due to the release of the suspended cells into the reactor. Consistent with expectations, bead integrity was breached due to the increase in cell numbers and reduction in bead stability through chemical and biological interactions with components within the effluent (Cruz et al., 2013). The beads which did not deteriorate retained form and function for a maximum period of 24 days at a 20 h HRT (Figure 4.6b). This is a significantly longer survival period than experienced by others. For instance, substantial degradation after 96 h was observed by Cruz et al. (2013).

Implementation of the technology would require that the cycle is terminated and the beads harvested prior to bead deterioration. The harvested beads can then be either applied to land as a fertiliser (Trejo et al., 2012) or converted into methane through anaerobic digestion as long as suitable pre-treatment is used (Ometto et al., 2014b). Additional consideration needs to be given to the reactor design to prevent washout of the calcium

phosphate precipitate, as the precipitated phosphate will continue to contribute to the total phosphate residual and further presents a useful resource for recovery.

4.4 Conclusions

- The intensification of the algal concentration afforded by the immobilisation process enables effective tertiary nutrient removal at contact times significantly lower than in alternative algal reactor systems. Effective removal to < 0.8 $mg.L^{-1}$ phosphate and <0.3 $mg.L^{-1}$ ammonia was observed at HRTs of 12 h (~ 4h bead contact time).
- Indirect removal through a pH induced precipitation process is a significant phosphate remediation pathway that is influenced by the relative abundance of ammonia and nitrate in the feed wastewater. Wastewaters high in nitrate and low in ammonia will result in substantial pH increase and corresponding calcium phosphate precipitation.
- The maximum operating rates for immobilised systems is defined through the phosphate loading rate with effective treatment up to a maximum loading rate of 0.8 $\mu gP.bead^{-1}d^{-1}$.
- The effective cycle time of operation is linked to the total phosphate treated with cycle times up to 24 days when operating at low loadings of 1.3 $g.m^{-3}.d^{-1}$ consistent with polishing effluent from 1 $mg.L^{-1}$ down to sub 0.3 $mg.L^{-1}$ at a HRT of 20 h (~6.7 h bead contact time).

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Photobioreactor.

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Chapter 5. The Effect of Light on Wastewater Nutrient Remediation by Immobilised Microalgae

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Abstract

Microalgae immobilised within a resin shaped into beads have demonstrated the ability to remediate nutrients from wastewater effluents within reasonable hydraulic retention times. Methods to further optimise performance consider parameters relating to the bead such as bead.mL^{-1}, cell stocking and bead size; with the impact of external conditions seldom investigated. Light is an essential parameter for microalgal growth with its effect on suspended cultures well documented. This work explores the influence of light on nutrient remediation by immobilised microalgae to determine whether similar relationships as with suspended cultures exist, or if the resin alters this relationship. The nutrient remediation performance of *Scenedesmus obliquus* immobilised in 2% calcium alginate was analysed under varying wavelengths and light regimes. The behaviours demonstrated by the immobilised biomass were similar to suspended cultures, however photoinhibition at photon flux densities (PFDs) as high at 1,000 µmol.m^{-2}.s^{-1} was not observed. The possibility of optimising the lighting through wavelengths, PFD and lighting regimes (i.e. flashing light and photoperiods) were analysed to explore the impact of the light regime on wastewater nutrient remediation by immobilised microalgae and a practical lighting solution recommended.

Keywords: flashing light, immobilisation, microalgae, photon flux density, photoperiod, wavelength

5.1 Introduction

Microalgae consume the macronutrients ammonium (NH_4^+) and phosphate (PO_4^{3-}), during their growth with the rate of uptake correlated to population growth (Xin et al., 2010). Accordingly, when sourced from wastewater effluents, microalgae based technology can be an effective approach for nutrient removal. As algae are photosynthetic organisms, reactor designs must ensure effective delivery of light to the algal biomass. The most common embodiment is a high rate algal pond (HRAP) which enables solar light penetration through the use of raceway ponds with water depths of 20-60 cm (Picot et al., 1992; Borowitzka, 1999; García et al., 2000) containing a relatively dilute biomass concentration of approximately 0.2 $gDW.L^{-1}$ (Craggs et al., 2012) consisting of a symbiotic community of microalgae and bacteria (Park and Craggs, 2010). As such, HRAPs are implemented at locations with a suitable annual climate enabling irradiation through solar radiation but resulting in a variation of photoperiod lengths (summer vs winter daylight hours) and light photon flux densities (PFDs, $\mu mol.m^{-2}.s^{-1}$) with the equivalent of approximately 700 to 1200 $\mu mol.m^{-2}.s^{-1}$ reported (Picot et al., 1992; García et al., 2000) with less than 50% of sunlight within the range of photosynthetically active radiation (PAR) (Walker, 2009). The need for shallow depths and dilute biomass concentrations in HRAPs results in the ponds needing to be operated at long hydraulic retention times of 4-10 days (Picot et al., 1992) with large associated footprints with design recommendations of 300-4,000 m^2 for individual ponds (Ben-Amotz, 2008).

Intensification of a microalgal based reactor, coupled with a reduction in reactor footprint, can be achieved through immobilising algal populations into alginate gels (Mallick, 2002) enabling hyper-concentration of algal biomass up to 3.3 $g(DW).L^{-1}$ (Chevalier and De la Noue, 1985) reported, with acceptable treatment reported when operating at hydraulic retention times (HRT) as low as 3 h (Chapter 4).

The PFD and wavelength (nm) are essential parameters for microalgal growth (Ugwu et al., 2008) and nutrient remediation and as such are recognised as key design features for microalgal bioreactors (Park and Lee, 2000; Lee and Lee, 2001). For instance, PFDs between 150 – 400 $\mu mol.m^{-2}.s^{-1}$ are reported for optimal growth of suspended *S.obliquus* (Liu et al., 2012; Gris et al., 2014), with higher PFDs resulting in photoinhibition. Light PFDs below this range (10 – 150 $\mu mol.m^{-2}.s^{-1}$) are described as light limiting and

correlated to a reduction in biomass productivity (Gris et al., 2014). Furthermore, microalgal chlorophyll molecules absorb light within the blue (450 nm) and red (650 – 700 nm) region of the spectrum most efficiently (Yeh and Chung, 2009) and improve the efficacy of photosynthesis. Suspended algae grown within single wavelength blue light are associated with increased phosphorus remediation (Kim et al., 2013), and improved nitrogen uptake through the activation of protein synthesis (Figueroa et al., 1995) and gene expression (Schulze et al., 2014), whereas red light is associated with increased specific growth rate (Wang et al., 2007).

In addition to PFD and wavelength, the antenna structure of the light harvesting complex of microalgae are unable to use all the photons absorbed in constant light (Park and Lee, 2000) and can store a pool of electrons for utilisation during dark periods (Vejrazka et al., 2015). Flashing light has been shown to improve the overall photosynthetic efficiency with increased biomass concentration of suspended *Chlorella vulgaris* observed under a flashing frequency of 37 kHz (Park and Lee, 2000) with similar growth profiles to constant light observed at a flashing frequency of <1kHz. Furthermore, a positive correlation has been observed between the length of the light period and nutrient remediation performance of microalgae (Lee et al., 2015) with 24 h lighting achieving an enhanced biomass concentration in comparison to 8 h for PFDs from 73 – 220 $\mu mol.m^{-2}.s^{-1}$ (Meseck et al., 2005).

Whilst work has been undertaken to optimise an immobilisation system in terms of cell stocking ($cells.bead^{-1}$), bead concentration ($beads.mL^{-1}$) and bead size (Abdel Hameed, 2007) the effect of lighting design and operation has not been as extensively evaluated nor optimised. Key aspects include (1) selecting the most appropriate wavelength and eliminating energy use on unwanted wavelengths (Yeh and Chung, 2009); and (2) reducing the lighting period through the use of on-off cycles (Matthijs et al., 1996) such as flashing and photoperiods. Accordingly, the current study aims to explore the impact of light regime on wastewater nutrient remediation by immobilised microalgae, understanding the translation from suspended to immobilised systems in order to propose recommendations for lighting regimes in the latter.

5.2 Materials and Methods

5.2.1 Microalgal cultivation and immobilisation procedure

The freshwater species *Scenedesmus obliquus* (276/42) was obtained from the Culture Collection for Algae and Protozoa (CCAP) (Oban, UK) and cultured in 50 L of Jarwoski Medium. Cultures were grown under a 24 hour light regime of approximately 100 – 150 $\mu mol.m^{-2}.s^{-1}$ at the culture surface which reduced to light limited conditions of < 50 $\mu mol.m^{-2}.s^{-1}$ for the lower half of the tank. As such, the culture was circulated under constant mixing by a circulation pump (900 $L.h^{-1}$) (Hydor Koralia Nano 900) to enable biomass exposure to light:limited light (dark) conditions of approximately 12:12 h. Biomass was harvested during the late exponential growth phase (after approximately 10 days) to enable maximum biomass recovery and stored overnight at 4^{o}C prior to immobilisation.

Beads were produced following the methods of Tam and Wong, (2000) and Ruiz-Marin et al., (2010). A sodium alginate (Na-alg) solution was mixed with concentrated algal biomass for a final Na-alg concentration of 2% and a final biomass concentration of approximately 10^5 $cells.bead^{-1}$ following bead production. The volume of algal concentrate required was calculated upon determination of the harvested cell concentration using a haemocytometer and light microscope (Olympus, BH Series) and the ability to produce approximately 4,000 beads (3 mm diameter) per 100 mL of algae-resin, estimated during preliminary trials.

The algae-resin solution was pumped by a peristaltic pump through tubing with a 2 mm diameter, capped with a 1 mL pipette tip and dripped into a 2% $CaCl_2$ solution from a height of 30 cm. The beads were left to solidify within the solution overnight and then washed several times with DI water to remove any surplus $CaCl_2$ prior to use.

The initial $cell.bead^{-1}$ concentration was confirmed by dissolving 10 beads within a known volume of 2% sodium citrate and determining the cell concentration ($cells.mL^{-1}$) using a haemocytometer and light microscope (Olympus, BH Series) in triplicate followed by back-calculating to confirm approximately 10^5 $cells.bead^{-1}$ during production. Blank beads were produced using the same methodology with no addition of algal biomass.

5.2.2 Wastewater

Secondary wastewater effluent was delivered weekly from a wastewater treatment plant (WWTP) located in the Midlands, UK with population equivalence (PE) of 32,000 which utilises an oxidation ditch for the main biological process. Wastewater was sampled from this site as the treated effluent is discharged into a catchment designated as a Site of Special Scientific Interest (SSSI). With implementation of the forthcoming water framework directive (WFD) across Europe, this WWTP will be targeted to meet a lower P residual concentration of 0.1 $mg.L^{-1}$. As such this WWTP is currently operating an advanced dosing regime with average wastewater characteristics during the experimental period summarised in Table 5.1. The collected effluent was supplemented with KH_2PO_4 and NH_4Cl to overcome the advanced upstream treatment and achieve consistent concentrations of approximately 1 $mg.L^{-1}$ PO_4-P and 2.5 $mg.L^{-1}$ NH_4-N. Wastewater was used upon delivery with the remainder stored at 4°C until use.

Table 5.1 Average wastewater characteristics over experimental period.

Parameter	Units	Value
PO_4-P	$mg.L^{-1}$	0.3[a]
NH_4-N	$mg.L^{-1}$	0.1[a]
NO_3-N	$mg.L^{-1}$	2.2
TSS	$g.L^{-1}$	0.1
COD	$mg.L^{-1}$	23.2
Alkalinity	$mg.L^{-1}$	132.8
pH		7.7

[a] Average concentrations prior to supplementation.

5.2.3 Experimental set up and light regime

Trials were run in batch within an Algem™ Labscale Photobioreactor (Stewartby, UK) (Figure 5.1) maintained at a constant temperature of 20°C. Conical flasks of 1 L were filled with 600 mL of wastewater effluent and a bead concentration of 10 $beads.mL^{-1}$ (Abdel Hameed, 2007) with an initial approximate biomass concentration of 1 $g(DW).L^{-1}$ of solely microalgal material. Reactors were mixed via a gimbal system at 120 rpm (Figure 5.1), with fluidisation of the beads confined to the lower third of the vessel simulating a packed bed configuration. Reactors were illuminated by an LED panel at the

base of the reactor over a surface area of 133 cm^2 and operated within the white (400 – 700 nm), blue (465 nm) and red (660 nm) spectra (Supplementary information, Appendix D; Figure A.3) at PFDs from 50 to 1000 $\mu mol.m^{-2}.s^{-1}$, representing a range of intensities considered as light limited to light saturated for suspended cultures.

A suspended biomass concentration of approximately 0.25 $mg(DW).L^{-1}$ was released by the beads during preliminary experiments lasting 24 h, representing approximately 0.03% of the total microalgal biomass within the reactor and as such considered negligible in the contribution to the overall microalgal nutrient remediation.

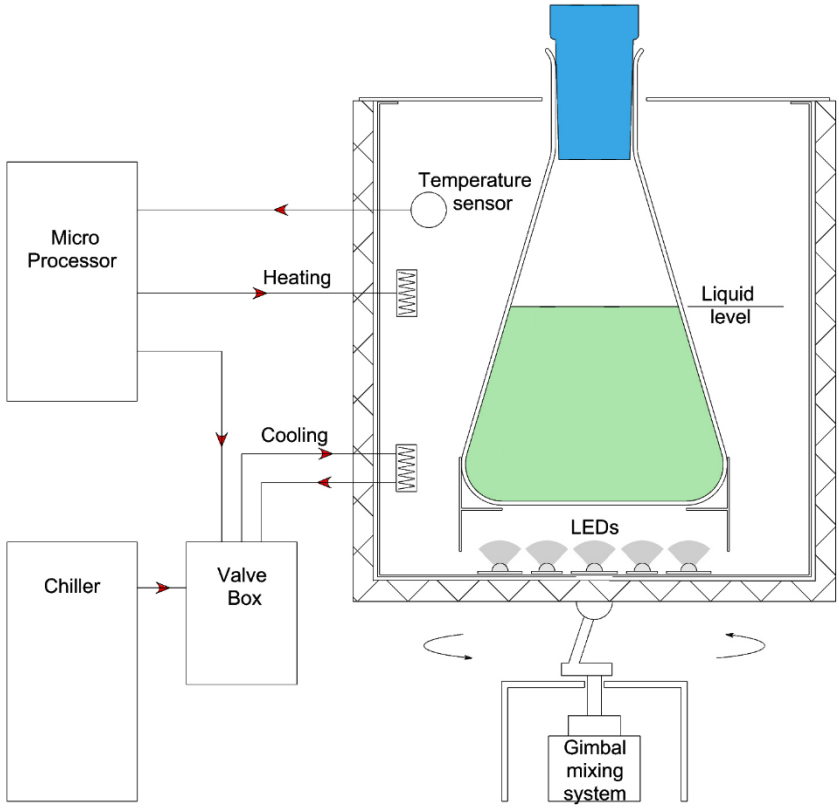


Figure 5.1 Schematic of the Algem™ Labscale Photobioreactor

Trials analysing performance at varying photoperiods were completed by programming the Algem™ to turn the LEDs on and off as necessary to create the required photoperiod length. For the flashing light trials, LEDs set at 200, 500 and 1000 $\mu mol.m^{-2}.s^{-1}$ were flashed at 238 Hz (maximum frequency possible at the time of the trial) at duty cycles between 25 – 100%. For information, 238 Hz is the equivalent to 0.004 seconds, at a duty

cycle of 75% the light:dark period would be 0.003:0.001 seconds with a 100% duty cycle the same as constant light.

5.2.4 Batch nutrient remediation sample analysis and biomass growth and yield on light energy

Samples of the treated effluent containing no microalgal beads were collected over a 24 h period. Analysis included pH, NH_4-N and PO_4-P with phosphate analysis including the total (tPO_4-P) and dissolved (dPO_4-P) fractions with insoluble phosphorus, characterising phosphate precipitation, determined through deducting the concentration of the dissolved fraction from the total concentration. Dissolved PO_4-P was analysed following syringe filtration at 0.45µm (Millipore, DE), whereas total phosphate (TP) was analysed using unfiltered samples. Residual P and N concentrations were analysed in duplicate using Spectroquant test kits (Merck Millipore) and read via a Spectroquant Nova 60 spectrophotometer and reported as the mean ± standard error when possible. Remediation performance was quantified as the reduction in the residual nutrient concentration, with removal associated to either direct uptake by the immobilised microalgae or precipitation/volatilisation facilitated by alkalisation of the local environment through biological activities of the microalgae.

Biomass growth within the beads was evaluated upon dissolving a sample of 10 beads within 2% sodium citrate and back-calculating the biomass concentration as previously described. Specific growth rate of the immobilised microalgae was then determined using Equation 5-1, where µ = specific growth rate (d^{-1}), $x1$ and $x2$ the cell.bead^{-1} concentration at time $t1$ and $t2$, reported as the mean ± standard error.

$$\mu = \frac{\ln(\frac{x1}{x2})}{t2 - t1} \qquad \text{Equation 5-1}$$

The biomass yield with light energy was calculated using Equation 5-2 as detailed in Zijffers et al. (2010), where Y = biomass yield (g.mol^{-1} photons), C = biomass concentration (g.L^{-1}), µ = specific growth date (d^{-1}), V = reactor volume (0.6 L), PFD = µmol.m^{-2}.s^{-1}, and A = illuminated surface area (0.0133 m^2). Biomass concentration (g.L^{-1}) was determined upon dissolving the beads to determine the cell concentration than estimating the biomass concentration from previous growth trials enabling a correlation between cell concentration and biomass concentration.

$$Y = \frac{C \times \mu \times V}{PFD \times A \times 86400 \times 10^{-6}}$$ Equation 5-2

5.2.5 Light transmittance

Light transmittance through the microalgal beads, blank beads and suspended biomass was completed to compare light attenuation and illumination depth. A suspended concentration of 10^6 cells.mL^{-1} was chosen as a cell concentration achieved during exponential growth through laboratory cultivation of *S.obliquus*. Suspended and immobilised samples were illuminated within the Algem™ at PFDs between 200 - 1000 µmol.m^{-2}.s^{-1} under the white, red and blue spectra. Light transmittance was measured in duplicate at the surface of the suspended sample and at five bead bed depths with a light meter (Apogee Quantum MQ-200 PAR Meter). The variation of light transmission (T) as a function of distance (l) in mm was calculated according to the Beer-Lambert law Equation 5-3 to determine the attenuation coefficient (a).

$$a = \frac{-logT}{l}$$ Equation 5-3

5.3 Results

5.3.1 Impact of wavelength and PFD on nutrient remediation and growth

The remediation rate under white light was observed to increase with increasing PFD for NH_4-N removal (Supplementary information Figure A.4, Figure 5.2a), from 26.4 to 30. 5 mgN.h^{-1}.10^{-6} beads for 50 to 1000 µmol.m^{-2}.s^{-1} respectively. Remediation of PO_4-P within white light at > 500 µmol.m^{-2}.s^{-1} demonstrated near complete exhaustion (< 0.15 mgP.L^{-1}) after 7 h (Figure 5.2b). In contrast, nutrient utilisation was lower under red and blue light which reached similar levels of remediation for PO_4-P after approximately 10 h. An increased uptake rate of 12.2 and 12.3 mgP.h^{-1}.10^{-6} beads was found for white light at 500 and 1000 µmol.m^{-2}.s^{-1} in comparison to 10.7 mgP.h^{-1}.10^{-6} beads for 50 and 200 µmol.m^{-2}.s^{-1} respectively (Figure 5.2a).

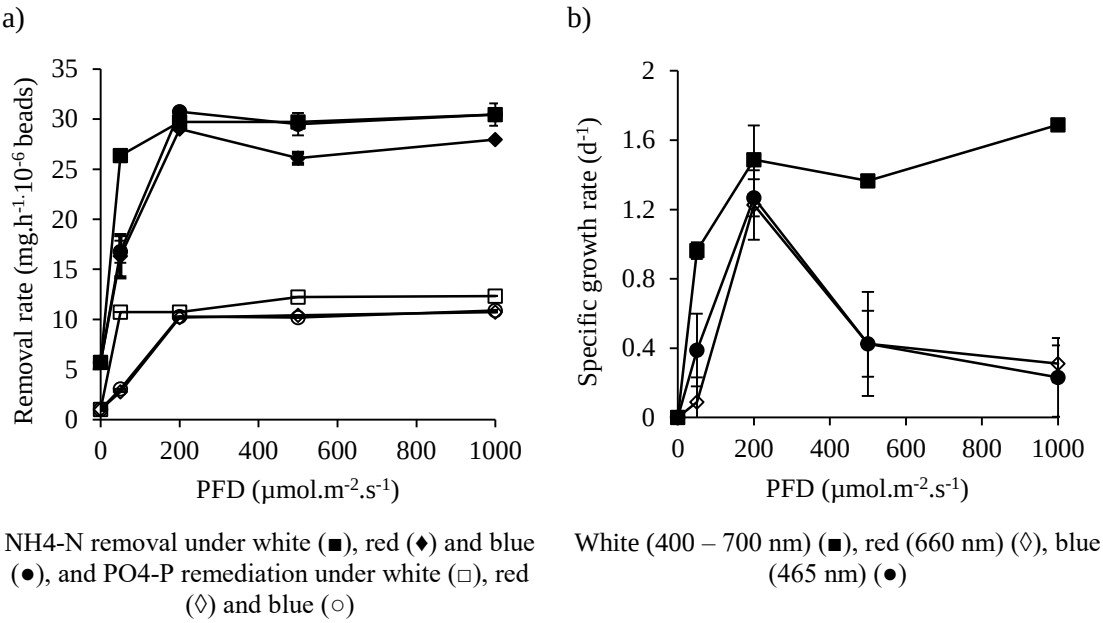


Figure 5.2 a) Nutrient removal rate and b) specific growth rate of immobilised microalgae for increasing PFDs and wavelengths (mean ± standard error).

Although enhanced performance was found with increasing PFD, the light utilisation efficiency associated to nutrient remediation was found to decrease from 146.8 µgN.mol^{-1} photon and 38.6 µgP.mol^{-1} photon at 50 µmol.m^{-2}.s^{-1} to 8.5 µgN.mol^{-1} photon and 2.2 µgP.mol^{-1} photon at 1000 µmol.m^{-2}.s^{-1} over the experimental period. The light energy supplied at the higher PFDs was therefore inefficiently utilised in the recovery of nutrients, with the lower PFDs demonstrating the greatest conversion of light energy into remediation capacity.

A similar relationship with PFD and increasing remediation rate was found under the red and blue light regimes (Figure 5.2a, SI Figure A.4). However, performance under 50 µmol.m^{-2}.s^{-1} was greatly reduced with a doubled NH_4-N residual and PO_4-P residual concentration six times greater than white light at 50 µmol.m^{-2}.s^{-1} after 10 h. Removal rates of 16.3 - 28.0 mgN.h^{-1}.10^{-6} beads and 2.8 - 10.7 mgP.h^{-1}.10^{-6} beads under the red light regime and 16.8 - 30.5 mgN.h^{-1}.10^{-6} beads and 3.1 - 10.9 mgP.h^{-1}.10^{-6} beads under the blue light regime were found after 10 h for 50 to 1000 µmol.m^{-2}.s^{-1} respectively, with a greater variation observed in the remediation performance of NH_4-N under the red light regime in comparison to white and blue light. Once again a decrease in the conversion of light energy into remediation ability was found for both wavelengths reducing from 61.4

$\mu gN.mol^{-1}$ photons [1] and 11.1 $\mu gP.mol^{-1}$ photons [1] under red light at 50 $\mu mol.m^{-2}.s^{-1}$ to 5.3 $\mu gN.mol^{-1}$ photons and 2.1 $\mu gP.mol^{-1}$ photons at 1000 $\mu mol.m^{-2}.s^{-1}$; and under blue light from 63.1 $\mu gN.mol^{-1}$ photons and 12.1 $\mu gP.mol^{-1}$ photons at 50 $\mu mol.m^{-2}.s^{-1}$ to 5.7 $\mu gN.mol^{-1}$ photons and 2.2 $\mu gP.mol^{-1}$ photons at 1000 $\mu mol.m^{-2}.s^{-1}$.

Overall, comparison of remediation under wavelengths revealed that white light demonstrated the greatest remediation rate for PO_4-P at all PFDs whereas for NH_4-N, blue and white light performed similarly at 200, 500 and 1000 $\mu mol.m^{-2}.s^{-1}$. Furthermore, the conversion of light energy to nutrient remediation was found to be greatest within a white light regime for all PFDs, with the greatest light conversion demonstrated at the lower PFDs. Those trials under no light exhibited minimal remediation with a reduction of 0.1 $mg.L^{-1}$ of PO_4-P and 0.4 $mg.L^{-1}$ of NH_4-N over a 10 hour period (SI, Figure A.4). The specific growth rate was negligible and demonstrated the importance of light for nutrient remediation by immobilised microalgae.

Microalgal growth was most consistently enhanced when irradiating the immobilised algae with white light compared to red or blue light across all tested PFDs, illustrated by specific growth rates of 0.96 ± 0.05, 0.39 ± 0.21 and 0.09 ± 0.04 for white, blue and red light respectively at an PFD of 50 $\mu mol.m^{-2}.s^{-1}$. The results are congruent with previous studies concerning a suspended culture of *S.obliquus* (Kim et al., 2013) and reflect that whilst chlorophyll molecules absorb light within the blue and red region of the spectrum most efficiently (Yeh and Chung, 2009) increased growth is expected under white light through adsorption of light across all the wavelengths that satisfy the pigment absorption bands (Matthijs et al., 1996) of the chlorophyll molecules. However, studies using immobilised *Chlorella vulgaris* reported enhanced growth from 0.38 d^{-1} to 0.81 d^{-1} upon switching from white to red light at an approximate PFD of 33 $\mu mol.m^{-2}.s^{-1}$ and 12 h HRT (Filippino et al., 2015) suggesting the impact may be species specific. However, the nutrient remediation performance was similarly enhanced under white light with ~80% NH_4-N removal and 100% PO_4-P (Filippino et al., 2015).

Across all wavelengths optimum growth occurred at an PFD of 200 $\mu mol.m^{-2}.s^{-1}$ which resulted in specific growth rates of 1.49 ± 0.2, 1.27 ± 0.11 and 1.23 ± 0.11 for white, blue and red light and biomass yields 0.72, 0.53 and 0.52 $g.mol^{-1}$ photons. Irradiating the immobilised algae at greater PFDs reduced the specific growth rate in the case of blue

and red light (Figure 5.2 b) with reduced biomass yields of 0.04 and 0.03 $g.mol^{-1}$ photons at 500 $\mu mol.m^{-2}.s^{-1}$ and 0.01 and 0.01 $g.mol^{-1}$ photons at 1000 $\mu mol.m^{-2}.s^{-1}$ for red and blue light respectively (consistent with a reduction in the conversion of light energy to nutrient uptake and associated biomass growth). In contrast, the specific growth was not reduced when irradiating at higher PFDs with white light such that the immobilised beads demonstrated no photo saturated inhibition which has been reported in the case of suspended cultures at PFDs > 150 $\mu mol.m^{-2}.s^{-1}$ (Gris et al., 2014). For instance, a reduction in biomass concentration of suspended *S.obliquus* from 1.2 x 10^8 to <8 x 10^7 $cells.mL^{-1}$ was observed when cultivated under white light at PFDs increasing from 150 to > 200 $\mu mol.m^{-2}.s^{-1}$ (Gris et al., 2014).

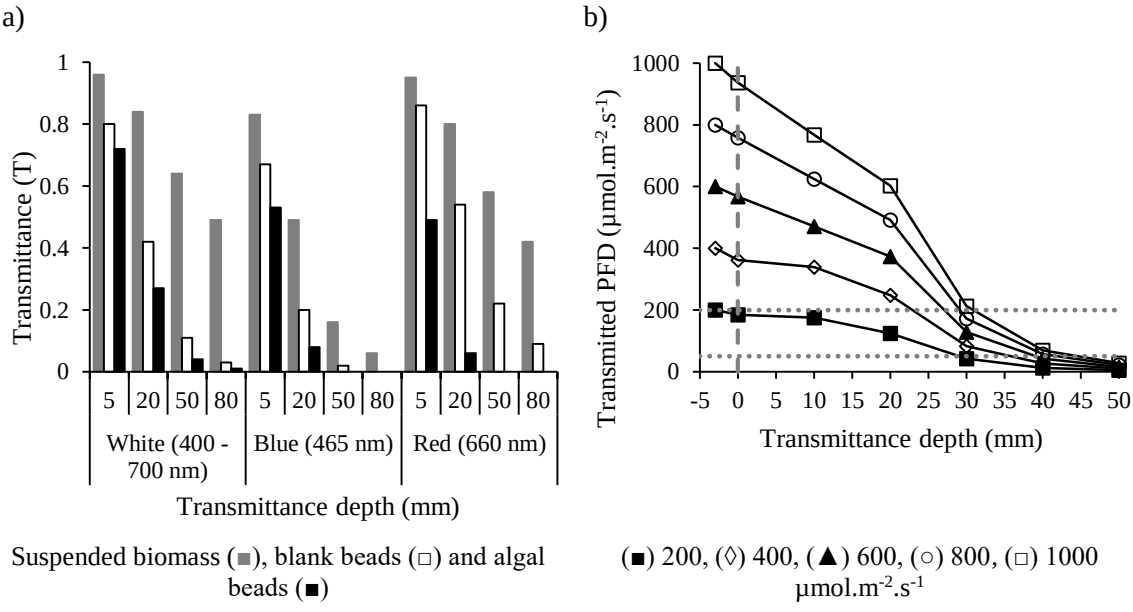
Remediation rates per bead averaged 29.7, 29.2 and 30.6 $mgN.h^{-1}.10^{-6}$ beads for white, red and blue light respectively at 200 $\mu mol.m^{-2}.s^{-1}$. The increased rate under blue light reflects similar total removal but reduced growth compared to white light congruent with its association with the activation of protein synthesis (Figueroa et al., 1995) and gene expression (Schulze et al., 2014) as well as the activation of the enzyme nitrate reductase (Kamiya and Saitoh, 2002). Similarities in nitrogen removal for a suspended culture of *S.obliquus* within white and blue light, despite a 45% increase in production rate within the white light regime have been previously demonstrated (Kim et al., 2013).

A pH increase of the localised environment was evident in all batch trials, from approximately 7.8 to 11.4. Red and blue light at 50 $\mu mol.m^{-2}.s^{-1}$ achieved a slightly reduced pH of 10.4. Alkalisation of the localised environment is a by-product of microalgal photosynthesis and as such, an indirect removal mechanism is likely to have contributed to NH_4-N remediation through volatilisation (though not directly measured) considering a pKa for ammonium of 9.51 at 20°C. The indirect removal of PO_4-P through precipitation was not found to take place as residual concentrations of tPO_4-P was similar to dPO_4-P throughout the trials.

5.3.2 Light attenuation

Light absorption by the beads containing algae was greater than the blank beads for all wavelengths, attenuating approximately 15, 14 and 48% more light than the blank beads at bed depths <20 mm for white, blue and red light respectively (Figure 5.3a). The increased reduction in transmission of red light in comparison to white and blue, for beads

containing biomass, is a function of the higher quantum efficiency by microalgae for photons within the red wavelength (Schulze et al., 2014) thereby preventing transmission depths seen within white and blue light. The red and blue wavelengths were absorbed within shorter distances by the immobilised biomass and reduced by almost 50% within 5 mm with no light beyond 50 mm. Transmission was better achieved by white light attaining a PFD of 50 µmol.m^{-2}.s^{-1} at a maximum bed depth of approximately 43 mm for the algal beads, in comparison to 39 and 24 mm for red and blue light at an initial PFD of 1000 µmol.m^{-2}.s^{-1} (data not shown). White light would therefore be considered the preferable lighting option, allowing the photoactive zone within an immobilised reactor to be larger in size when applied to full scale treatment in comparison to a reactor lit solely by a blue or red light regime. In comparison, the light transmission through the suspended biomass penetrated greater depths when compared to the immobilised biomass (Figure 5.3a), with calculated maximum depths of 325, 260 and 65 mm attaining a PFD of 50 µmol.m^{-2}.s^{-1} for white, red and blue respectively (data not shown) limiting the depth of suspended systems such as open ponds to maximum depths of approximately 30 cm.



Suspended biomass (■), blank beads (□) and algal beads (■)

(■) 200, (◊) 400, (▲) 600, (○) 800, (□) 1000 µmol.m^{-2}.s^{-1}

Figure 5.3 a) Light transmittance at 1000 µmol.m^{-2}.s^{-1} for white, blue and red wavelengths and; b) light transmittance depth through a bed of beads in relation to PFD with (--) denoting the base of the Pyrex conical flask and initial reduction in PFD and (..) the critical PFD band required for activity.

The implications of light attenuation was further analysed for an immobilised system to profile the reduction in white light transmittance of increasing PFDs within a packed bed

of algal beads. A similar pattern of attenuation was observed for all PFDs with an initial reduction of approximately 20% within the first 10 mm, increasing to > 98% at depths > 50 mm (Figure 5.3b). Operation at different PFDs altered the depth the light was able to reach and thus controlled the size of the photoactive volume within the reactor. A target level of 50 $\mu mol.m^{-2}.s^{-1}$ is known to maintain effective activity (Figure 5.3b) (Liu et al., 2012; Gris et al., 2014) which was delivered to bed depths of 20.9, 32.0, 38.5, 42.9 and 46.2 mm for PFDs of 200, 400, 600, 800 and 1000 $\mu mol.m^{-2}.s^{-1}$ respectively. Irradiating the beads at PFDs beyond 200 $\mu mol.m^{-2}.s^{-1}$ was not observed to increase growth or substantially enhance nutrient remediation, with the excess photons wasted as refracted light or heat (Park and Lee, 2000) with approximately 40% of light unutilised by the microalgae when supplied at 400 $\mu mol.m^{-2}.s^{-1}$ and 80% for 600, 800 and 1000 $\mu mol.m^{-2}.s^{-1}$ respectively, with light conversion efficiencies into nutrient remediation previously demonstrating no further benefit when illumination at the greater PFDs. In comparison, all the light supplied at 200 $\mu mol.m^{-2}.s^{-1}$ could be utilised by the microalgae as the light supplied was within the critical PFD band. The total bed depth within the critical PFD band of 50 to 200 $\mu mol.m^{-2}.s^{-1}$ is estimated as 20.9, 21.3, 21.5, 21.4 and 21.3 mm for starting PFDs of 200, 400, 600, 800 and 1000 $\mu mol.m^{-2}.s^{-1}$ respectively (Figure 5.3b) with initial PFDs of between 200 - 400 $\mu mol.m^{-2}.s^{-1}$ recommended to overcome the initial attenuation of the glass reactor and enable effective use of the total supplied light and a greater conversion of the provided light energy to nutrient remediation (demonstrated at lower PFD).

5.3.3 Light regimes

5.3.3.1 Flashing and constant light

The impact of the time-averaged PFD on specific growth rate of the immobilised *S.obliquus* demonstrates a characteristic growth curve with increasing PFD, modelled by the immobilised biomass within the constant and flashing light regimes (Figure 5.4a). This is consistent with the concept of partial light integration, where productivity under a flashing light is consistent with productivity under constant light, typically observed at flash frequencies > 1 Hz for suspended cultures (Jr and Myers, 1954). During partial light integration scenarios, microalgae productivity is relative to the time-averaged PFD which incorporates the dark period and not the actual PFD (Vejrazka et al., 2015); for example

a 75% duty cycle with a L:D of 0.003:0.001 second at 500 µmol.m^{-2}.s^{-1} equates to a PFD of 375 µmol.m^{-2}.s^{-1}. Specific growth under time-averaged PFD through flashing in comparison to constant light of the same PFD demonstrated a reduced specific growth rate (consistent with partial light integration), for instance a specific growth rate of 0.96 (± 0.05) d^{-1} in comparison to 0.47 (± 0.28) d^{-1} within constant light and flashing light averaging 50 µmol.m^{-2}.s^{-1} (Figure 5.2a, Figure 5.4a) and 1.6 (± 0.37) d^{-1} and 0.9 (± 0.21) d^{-1} for constant light of 200 µmol.m^{-2}.s^{-1} and flashing light averaging 250 µmol.m^{-2}.s^{-1} (Figure 5.4a). The highest specific growth rates of 2.2 and 2.6 d^{-1} during the trials were found under the flashing light regime of a time-averaged PFD of 375 and 750 µmol.m^{-2}.s^{-1}.

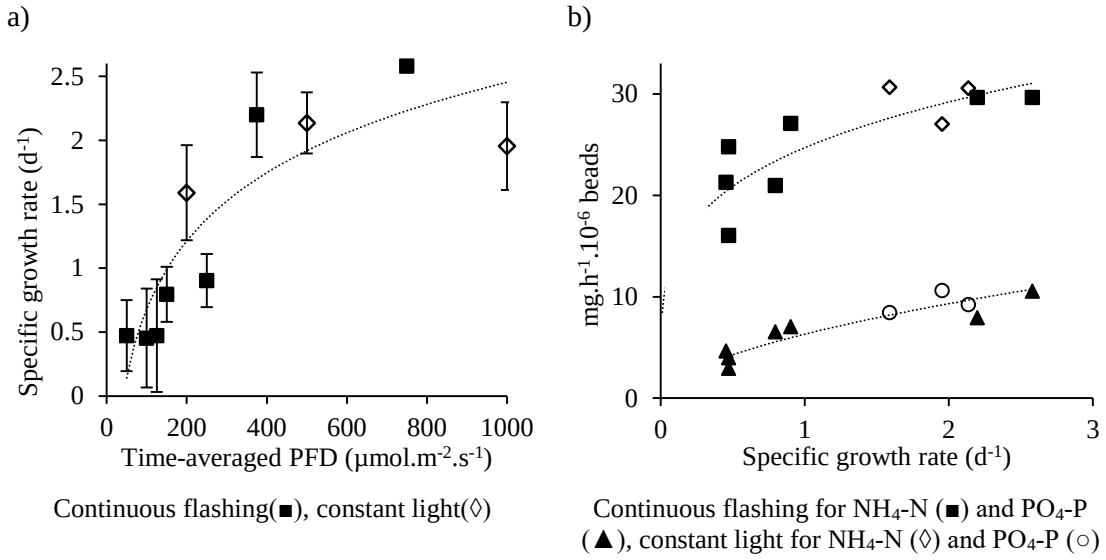


Figure 5.4 a) Effect of time-averaged PFD on specific growth rate, and; b) phosphate and ammonium bead uptake rate and specific growth rate.

As expected an increase in specific growth rate of the immobilised microalgae correlated to an increase in the bead uptake rate with uptake rates averaging 3.9 mgP.h^{-1}.10^{-6} beads and 20.7 mgN.h^{-1}.10^{-6} beads at the lowest observed growth rate under flashing light of 0.5 d^{-1} in comparison to 10.5 mgP.h^{-1}.10^{-6} beads and 30.0 mgN.h^{-1}.10^{-6} beads at a the maximum growth rate of 2.6 d^{-1} (Figure 5.4b).

The light energy required in mols of photons can be calculated depending on the PFD, illumination surface area and the required treatment period to obtain <0.1 mgP.L^{-1} for constant light and flashing light using Equation 5-4 and Equation 5-5, where PFD = µmol.m^{-2}.s^{-1}, A = illuminated surface area (m^2), t_t= length of treatment period (h); and

for flashing light where f_l = length of the flash period (seconds) and f_d= length of the dark period (seconds).

$$mols\ photons\ (constant)\ = PFD \times A \times t_t \times 3.6 \qquad \text{Equation 5-4}$$

$$mols\ photons\ (flashing) = PFD \times A \times \left(\frac{f_l}{f_l + f_d}\right) \times t_t \times 3.6 \qquad \text{Equation 5-5}$$

The energy requirement for all the lighting scenarios (constant and flashing) are outlined within Table 5.2. The lowest energy requirement of 57.5 mols photons is demonstrated by a 200 µmol.m^{-2}.s^{-1}at a 25% duty cycle, however the consequence of a reduction in energy is an extended treatment period of 24 h. The regime with the shorter treatment period of 7 h was demonstrated at the 1000 µmol.m^{-2}.s^{-1}, at constant and 75% duty cycle with the duty cycle reducing the energy used by 83.8 mols photons over the treatment period but with energy requirements x6 and x4 greater than that required by the regime with the lowest photon energy demand (Table 5.2). The lighting regime selected is therefore a trade-off between treatment period (reactor size) and energy requirements.

Table 5.2 Light energy requirements (mols photons) and treatment time for constant and flashing light regimes, ordered by increasing energy requirement.

	PFD, duty cycle (%)	t_t	**mols photons (increasing)**
Constant	200, 100	24	229.8
	1000, 100	7	335.2
	500, 100	24	574.6
Flashing	200, 25	24	57.5
	200, 50	24	114.9
	1000, 25	10	119.7
	500, 25	24	143.6
	200, 75	24	172.4
	1000, 50	10	239.4
	1000, 75	7	251.4
	500, 50	24	287.3
	500, 75	24	430.9

5.3.3.2 Photoperiods

The nutrient remediation performance of the immobilised *S.obliquus* during the photoperiod trials found remediation of PO_4-P to <0.1 $mg.L^{-1}$ following 18, 18, 18 and 12 h for photoperiods of 1.5:1.5, 3:3, 6:6 and 12:12 h L:D respectively for 1000 $\mu mol.m^{-2}.s^{-1}$, whereas remediation < 0.1 $mgP.L^{-1}$ under 200 $\mu mol.m^{-2}.s^{-1}$ only occurred following 24 and 18 h for the 6 and 12 h L:D regime (Figure 5.). The remediation of NH_4-N to < 0.1 $mg.L^{-1}$ for all light regimes occurred within shorter treatment periods than PO_4-P of 16.5, 21, 18 and 12 h for 200 $\mu mol.m^{-2}.s^{-1}$ and 13.5, 15, 18 and 12 h for 1000 $\mu mol.m^{-2}.s^{-1}$ for 1.5:1.5, 3:3, 6:6 and 12:12 h L:D respectively (Figure 5.).

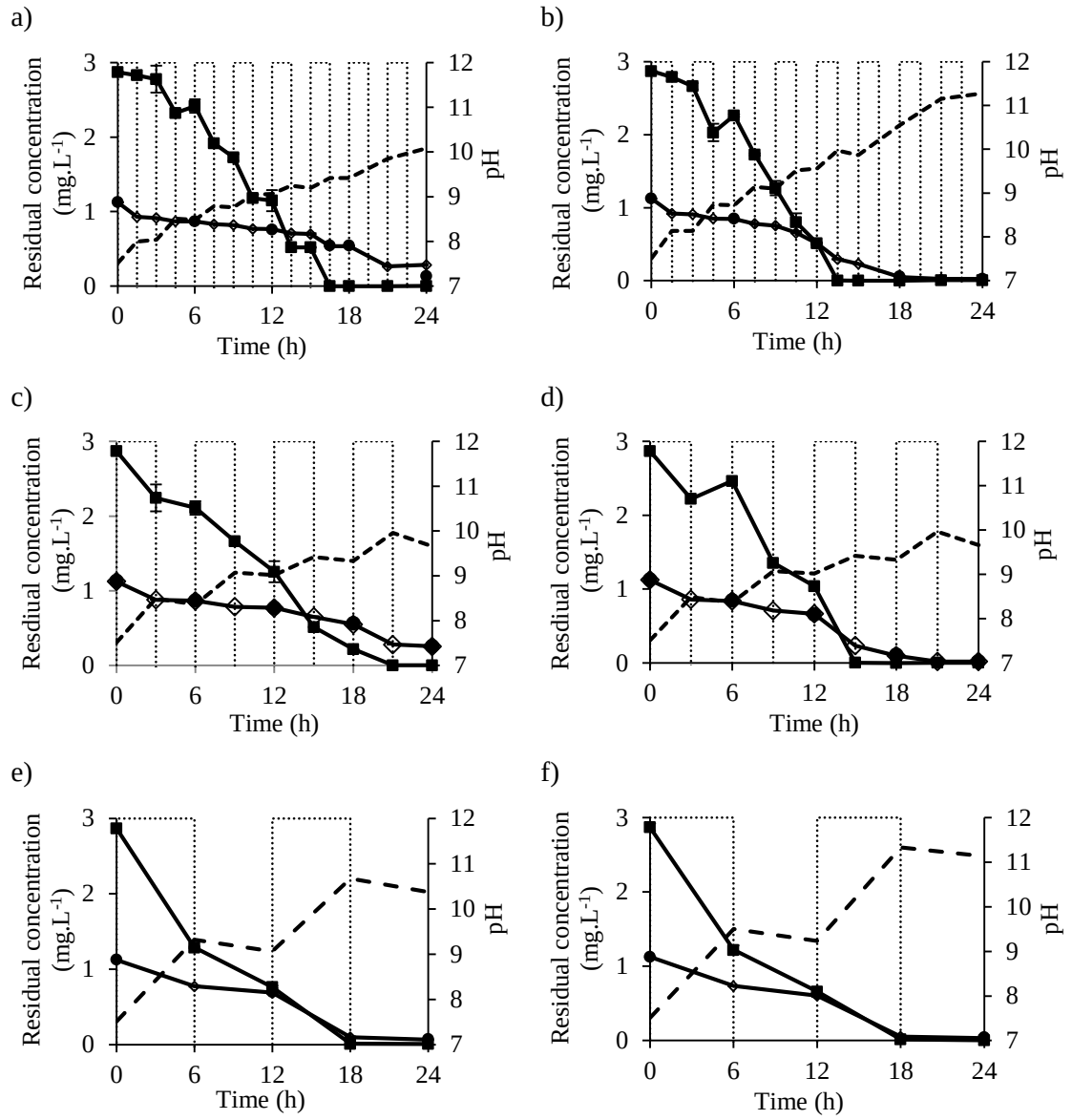


Figure 5.5 Photoperiod trials with light:dark regime of 1.5:1.5 h for a) 200 µmol.m^{-2}.s^{-1}, b) 1000 µmol.m^{-2}.s^{-1}, 3:3 h for c) 200 µmol.m^{-2}.s^{-1} and d) 1000 µmol.m^{-2}.s^{-1}, 6:6 h for e) 200 µmol.m^{-2}.s^{-1} and f) 1000 µmol.m^{-2}.s^{-1}. (■) NH_4-N, (◊) dPO_4-P (▲) tPO_4-P and (- -) pH. Dashed profile denotes photoperiod, sections with top border representing the light period.

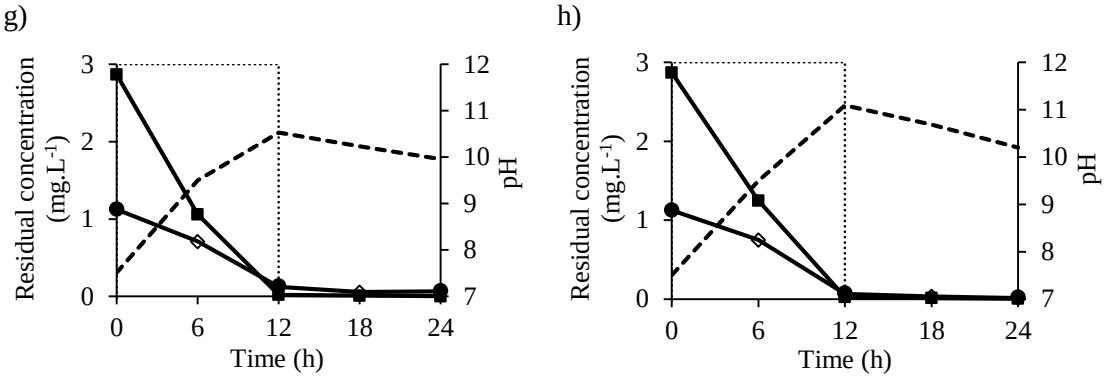


Figure 5.5 continued.. Photoperiod trials with light:dark regime of 12:12 h for g) 200 µmol.m^{-2}.s^{-1} and h) 1000 µmol.m^{-2}.s^{-1}. (■) NH_4-N, (◊) dPO_4-P (▲) tPO_4-P and (- -) pH. Dashed profile denotes photoperiod, sections with top border representing the light period.

When taking into consideration the length of the treatment period necessary for remediation to < 0.1 mg.L^{-1} for phosphate, the remediation rate within the light episodes for 200 µmol.m^{-2}.s^{-1} increased from 5.4 to 8.3 mgP.h^{-1}.10^{-6} beads for 1.5:1.5 to 12:12 h L:D regime and 16.9 to 23.7 mgN.h^{-1}.10^{-6} beads for NH_4-N (Table 5.3). Bead remediation rates were generally found to increase, however upon consideration of the standard error, the mean remediation rate for all lighting periods can be considered similar.

Likewise, remediation during the corresponding dark episodes increases with increasing length of dark period from 0.3 to 1.0 mg.h^{-1}.10^{-6} beads for 1.5:1.5 to 6:6 h, equivalent of 6 – 13% of the remediation rate during the light episodes (Table 5.3), though can be considered equal in performance when considering mean standard error. Minimal uptake was observed within the dark episode of the 12:12 regime through remediation to approximately 0.1 mg.L^{-1} within the initial 12 h of light.

Table 5.3 PO_4-P and NH_4-N removal rate during the light and dark episodes, considering the total treatment period for photoperiods of 1.5:1.5, 3:3, 6:6 and 12: 12 L:D (mean ± standard error).

L:D	µmol. $m^{-2}.s^{-1}$	PO_4-P removal rate ($mg.h^{-1}.10^{-6}$ beads)				NH_4-N removal rate ($mg.h^{-1}.10^{-6}$ beads)			
		1.5:1.5	3:3	6:6	12:12	1.5:1.5	3:3	6:6	12:12
Light	200	5.4 (1.9)	6.0 (1.5)	7.8 (2.0)	8.3 (1.4)	--	16.9 (3.8)	19.4 (6.9)	23.7 (6.4)
	1000	9.7 (2.2)	9.3 (5.2)	7.8 (1.3)	8.8 (2.6)	29.7 (6.4)	35.8 (1.2)	19.1 (8.4)	23.7 (3.3)
Dark	200	0.3 (0.2)	0.7 (0.7)	1.0 (0.4)	0.5 (0.6)	1.7 (2.1)	3.0 (3.0)	4.4 (4.4)	0.2 (0.0)
	1000	1.9 (1.3)	1.0 (1.0)	1.3 (0.9)	0.4 (0.1)	6.1 (5.1)	3.8 (3.8)	4.8 (4.6)	0.2 (0.0)

An alternative remediation behaviour was initially demonstrated for immobilised *S.obliquus* within the 1000 µmol.$m^{-2}.s^{-1}$ regime, with a decrease in the mean PO_4-P remediation rate from 9.7 to 8.8 mgP.$h^{-1}.10^{-6}$ beads with an increasing photoperiod length from 1.5:1.5 to 12:12 h L:D, with a similar behaviour observed during the dark period of 1.9 to 1.3 mgP.$h^{-1}.10^{-6}$ beads for dark episode lengths of 1.5:1.5 to 6:6 h L:D (Table 5.3) representing 11 – 20% of the remediation rate during the light episode. However, when factoring standard error, remediation rates during all light and dark periods for PO_4-P and dark periods of NH_4-N remediation performed similarly, with notable increases in performance only for NH_4-N under 1000 µmol.$m^{-2}.s^{-1}$ in lighting periods increasing from 1.5:1.5 to 3:3 (L:D) followed by lower yet similar remediation rates at the longer lighting periods of 6:6 and 12:12 (L:D).

Continued remediation of both PO_4-P and NH_4-N by the immobilised microalgae during the dark period is possible through storage of electrons supplied through light (Vejrazka et al., 2015) for the creation of the chemical energy ATP (adenosine triphosphate) within the light dependant reactions of photosynthesis. The ATP produced during the light period is subsequently utilised during the light independent reaction (Calvin Cycle) in the conversion of CO_2 to glucose and the anabolism of amino acids and nucleotides from the assimilated NH_4-N and PO_4-P into proteins and nucleic acids for growth. The creation and availability of ATP from the light dependant reaction enables a reduced rate of uptake to continue during the dark period, providing sufficient light has been provided during

the light episodes. As such, 1000 µmol.m^{-2}.s^{-1}at a 1.5:1.5 L:D regime creates sufficient ATP during the light episode to sustain remediation through the length of the dark period (albeit at a reduced rate), with the highest corresponding removal rate within the light episode also seen for the 1.5:1.5 h L:D regime and similar to the bead remediation rate observed under constant light of 10.6 mgP.h^{-1}.10^{-6} beads and 30.7 mgN.h^{-1}.10^{-6} beads (Figure 5.4b).

A longer L:D photoperiod length was required when illuminating at 200 µmol.m^{-2}.s^{-1} to generate sufficient ATP to enable activity during the dark episode, with increases in maximum uptake rates during the light and dark episodes corresponding with a 6:6 – 12:12 h L:D regime similar to those observed under constant light of 8.5 mgP.h^{-1}.10^{-6} beads and 27 mgN.h^{-1}.10^{-6}beads (Figure 5.4b).

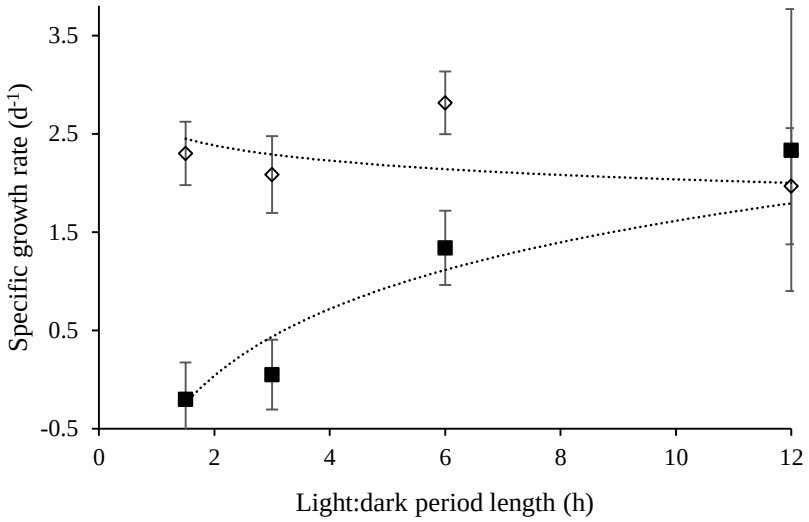


Figure 5.6 Impact of photoperiod on specific growth rate. (■) 200 µmol.m^{-2}.s^{-1} and (◊) 1000 µmol.m^{-2}.s^{-1}.

Alteration of the L:D period during the 1000 µmol.m^{-2}.s^{-1} trial resulted in a similar specific growth rate of 2.3 (± 0.32) d^{-1} at a photoperiod of 1.5:1.5 h L:D and 1.97 (± 0.59) d^{-1} for 12:12 photoperiod (24 h L:D) (Figure 5.6). In contrast, the specific growth rate under 200 µmol.m^{-2}.s^{-1} increased from negligible growth (-0.2 d^{-1}) at a photoperiod of 1.5:1.5 h L:D to a maximum of 2.3 (±1.4) d^{-1} at a photoperiod of 12:12 h L:D. Similar behaviour was found by Meseck et al. (2005) for a suspended culture of *Tetraselmis Chui* at 200 µmol.m^{-2}.s^{-1} and was concluded that biomass production at a specific PFD was

dependent upon the length of photoperiod. Meseck et al. (2005) did not analyse performance at PFDs higher than 200 $\mu mol.m^{-2}.s^{-1}$, but suggested high PFDs did contribute to a higher biomass production and coupled utilisation of nutrients. As such, enhanced levels of remediation can be achieved through photoperiods of illumination at 200 or 1000 $\mu mol.m^{-2}.s^{-1}$ by simulating a photoperiod of 6:6 and 1.5:1.5. These lighting scenarios equate to an energy requirement of 114.9 and 430.9 mols photons respectively (calculated through Equation 5-5), in comparison to 229.8 and 335.2 mols photons necessary for treatment under a constant 200 and 1000 $\mu mol.m^{-2}.s^{-1}$ lighting regime. The required treatment time (18 h) is the same for both scenarios for 200 $\mu mol.m^{-2}.s^{-1}$ and as such, using a 6:6 h profile enables a 50% reduction in energy requirements.

5.4 Discussion: Implications for lighting microalgae for wastewater nutrient remediation

The aim of the study was to examine the effect of lighting regime on the performance of immobilised *S.obliquus* in the remediation of wastewater nutrients to enable recommendations of a suitable light profile for effective performance for each lighting regime. As such, when considering constant light, flashing light or photoperiods; a series of recommendations for optimal performance can be made for an immobilised system in comparison to those for a suspended system (Table 5.4).

Table 5.4 Suggested light regimes for immobilised algal systems (from this study) in comparison to suspended systems for productivity and nutrient remediation.

Light regimes	Immobilised *S.obliquus* (this study)	Suspended microalgae	Reference
Wavelength	White	White (growth) Blue (N removal)	(Kim et al., 2013)
PFD	200 – 400	150 – 400	(Liu et al., 2012; Gris et al., 2014;),
Duty cycle (%) (Frequency)	50/75 (238 Hz)	< 50% (> 2.5 kHz)	(Park and Lee, 2000; Zhang et al., 2015).
Photoperiod L:D (h)	1.5:1.5 at 1000 $\mu mol.m^{-2}.s^{-1}$ 6:6 at 200 $\mu mol.m^{-2}.s^{-1}$	24:0 at 73 – 220 $\mu mol.m^{-2}.s^{-1}$	(Meseck et al., 2005)

Biomass productivity and associated nutrient remediation was enhanced under white light for immobilised microalgae, with a similar preference demonstrated for suspended microalgae for growth through the inclusion of the blue and red wavelengths necessary for essential metabolic processes correlated to nutrient uptake and growth.

Immobilised microalgae were found to withstand light intensities of up to 1000 $\mu mol.m^{-2}.s^{-1}$ with no evidence of photoinhibition. However no substantial increase in remediation performance was demonstrated in relation to the increased PFD, with a removal efficiency similar to that demonstrated at a lower PFD of 200 $\mu mol.m^{-2}.s^{-1}$. Furthermore, profiles for light attenuation with depth for the immobilised beads found only minor increases in the depth of the bed subjected to the range of 'critical PFDs' of between 50 – 200 $\mu mol.m^{-2}.s^{-1}$. As such, illuminating at intensities beyond 400 $\mu mol.m^{-2}.s^{-1}$ can be concluded not to provide any additional benefit, with unutilised photons wasted as heat and light. When considering performance and attenuation depth of a packed bed of beads, a PFD of between 200 – 400 $\mu mol.m^{-2}.s^{-1}$ appears beneficial both in terms of performance and the ability to maintain all the beads within the 'critical PFD' throughout the illuminated bed depth. Alternative reactor options with reduced bead concentrations and bead agitation through fluidisation would enable an increased attenuation depth. Overall, findings for a packed bed of beads are similar to the recommended PFD for suspended *S.obliquus* (Table 5.4), however the limit for the suspended system is a result of photoinhibition.

Performance under a flash frequency of 238 Hz was found to perform similarly to constant light at a PFD of 500 $\mu mol.m^{-2}.s^{-1}$ at a 50 and 75% duty cycle and 1000 $\mu mol.m^{-2}.s^{-1}$at a 75% duty cycle and as such provides a lighting regime which offers a 25 – 50% reduction in lighting period and light demand of 251.4 – 430.9 mols photons. However, constant light at 200 $\mu mol.m^{-2}.s^{-1}$ was found to perform similarly to flashing 500 and 1000 $\mu mol.m^{-2}.s^{-1}$ at a light demand of 229.8 mols photos, offering a further 8.6 – 46.7% reduction in light requirements through a reduction in photon flux and preferable over a flashing light regime of a higher PFD. Furthermore, the reduced specific growth rate observed under constant light can be considered beneficial for an immobilised system through prolonging the bead life prior to biomass breakthrough, inevitable within an immobilised system due to the finite capacity of the bead.

The use of photoperiods at shorter L:D regimes for high PFDs and longer L:D regimes for lower PFDs enables remediation during the light episodes equivalent to that of constant light (Table 5.4). However, the reduced remediation performance during the dark periods extends the overall treatment period in comparison to constant light. For example, the 1000 µmol.m^{-2}.s^{-1} PFD at a photoperiod of 6:6 enabled remediation to <0.1 mgP.L^{-1} following 18 h in comparison to approximately 7 h under a constant lighting system, thereby more than doubling the required treatment period when using a 50% photoperiod. However, photoperiods are not solely applied through turning lights on and off, but can be incorporated into a reactor design such that the biomass is circulated or mixed within a reactor so the time spent within the light and dark mimic that of a photoperiod. A large scale bioreactor necessary for full scale treatment could therefore be designed to enable half of the contained biomass to be illuminated and circulation every 6 h reducing the ancillary lighting equipment necessary and associated operational expenditure, offering a trade-off between the size of the reactor and lighting regime to be considered during design and delivery.

Overall observations have found an intensity of approximately 300 – 400 µmol.m^{-2}.s^{-1}, constant white light to achieve enhanced remediation performance by immobilised *S.obliquus* for the treatment of wastewater nutrients.

5.5 Conclusions

- The relationship between microalgae and light is mostly unaffected by immobilisation, with immobilised biomass withstanding greater PFDs through light attenuation by the bead resin.
- White light demonstrated increased remediation performance by immobilised *S.obliquus* than that under a red or blue wavelength.
- A PFD no greater than 300 – 400 µmol.m^{-2}.s^{-1} is suitable when irradiating a packed bed of immobilised microalgae as higher intensities were found not to improve light attenuation depth of the ‘critical PFD’ of 50 – 200 µmol.m^{-2}.s^{-1} when considering a packed bed reactor.

- Reducing light periods through flashing and photoperiods reduced the light energy demand but extended the overall treatment time, with constant light determined as the optimal lighting regime.

5.6 Acknowledgements

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Chapter 6. Understanding the Implementation Challenges of Using an Immobilised Microalgal Bioreactor for the Remediation of Wastewater Nutrients: An Economic Assessment

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Abstract

Forthcoming changes in the Water Framework Directive will challenge water utilities to further reduce their phosphorus discharges to <0.5 $mgP.L^{-1}$. Whilst conventional treatment options are able to meet the new consents, they often represent a linear economy where resources are unrecovered following treatment e.g. coagulation. The aspiration is to move towards solutions with wider environmental benefits which contribute to the delivery of a circular economy. One such novel solution which has gained interest is immobilised microalgae. This technology is currently within its infancy in relation to wastewater remediation and believed an expensive alternative when applied at full scale. This paper undertakes an economic assessment on an immobilised microalgal bioreactor (IBR) in light of recent research to optimise operational parameters, to identify key challenges in implementation through CAPEX, OPEX and whole life cost (WLC) estimates. Initial findings find present day implementation of an IBR to be economically viable providing a supply chain for immobilised microalgae is available. Further cost reductions predicted within the next 10 years estimates an IBR to be an energy positive process with a favourable WLC in comparison to conventional solutions.

Keywords: microalgae, immobilisation, wastewater, nutrient remediation, OPEX, CAPEX, WLC, optioneering analysis, water framework directive

6.1 Introduction

Wastewater treatment plants (WWTP) within the United Kingdom (UK) are regulated by the Urban Wastewater Treatment Directive (UWWTD) to meet prescribed effluent consents prior to discharge to a receiving catchment. Discharge of nutrients, namely

phosphorus (P) and nitrogen (N), play a pivotal role in the receiving water's condition as an enrichment of P and N contribute to eutrophication and a decline in the water body quality. As the limiting nutrient commonly associated as the cause of eutrophication (Smith et al., 1999), the introduction of P into the receiving water body is limited to 2 $mgP.L^{-1}$ for a WWTP treating a population equivalent (PE) of 10,000 – 100,000 and 1 $mgP.L^{-1}$ for a PE >100,000 (European Community, 1991) with considerable investment by water utilities to meet UWWTD consents, typically through dosing a metal coagulant for the precipitation of P and removal following filtration and/or settlement.

Recently, there has been renewed interest in alternative technologies to further reduce P discharges through the implementation of the Water Framework Directive (WFD). The WFD will challenge water utilities to reduce final P concentrations beyond the limits prescribed within the UWWTD with the primary aim of protecting the receiving water body, regardless of the scale of operation, to below 0.5 $mgP.L^{-1}$. With some WWTPs, such as those discharging to Sites of Special Scientific Interest (SSSI), expected to meet final effluent concentrations of 0.1 $mgP.L^{-1}$ (Mainstone and Parr, 2002; Jarvie et al., 2006).

Whilst conventional treatment strategies (i.e. coagulation) are able to meet the enhanced WFD targets, its implementation at WWTP which do not currently incorporate chemical dosing (such as small rural works of PE < 2,000, which characterise the majority of WWTP within the UK, (Upton et al., 1995)) poses a challenge through; 1) the need of improved infrastructure for the transport of chemicals and residual sludge on and off site, 2) chemical storage facilities and 3) health and safety (H&S) facilities including safety showers supplied with potable water (Germain-Cripps, 2016). Furthermore, coagulation strategies are characteristic of a linear economy model where resources are unrecovered and disposed, with 63% of the total sludge produced in Europe incinerated or landfilled (European Commission, 2009). The aspiration is to move towards solutions which offer wider environmental benefits through energy generation, resource recovery and/or material cascading and in so doing align to the concept of a circular economy model. Alternative solutions which can be retrofitted to current WWTP, further polish secondary/final effluent to meet the discharge limits within the WFD and contribute to the circular economy are therefore desirable. Accordingly there is considerable

investment by water utilities in the research and development of potential technologies for implementation of such technologies within the next 5 – 10 years to coincide with the next AMP (Asset Management Plan) cycle (Vale, 2016). Options being considered include a number of variants utilising coagulant with different down-stream clarification technologies such as cloth filters (Mecana), membranes, depth filters (BluePro) and ballasted coagulation (Co-Mag) (Vale, 2016). In addition a number of more innovative solutions are under development that incorporate opportunities in relation to delivery of a circular economy concept such as nanoparticle embedded ion exchange (resource recovery) (Vale, 2016) and reactive media for use in constructed wetlands (material cascading) (Germain-Cripps, 2015). All the above utilise chemical pathways and thus interest remains in options based around biological pathways such as those delivered through the use of microalgae.

Microalgae are ubiquitous in wastewater environments (Davis et al., 1990) and assimilate P and N within the effluent during their growth (Chiu et al., 2015; Christenson and Sims, 2011). Furthermore, microalgae can contribute to the circular economy through biogas production providing a suitable pre-treatment prior to anaerobic digestion is incorporated (Ometto, 2014). Uptake of microalgal systems for wastewater nutrient remediation are however lacking in the UK through limited knowledge of operation within the UK climate, and the belief that such a solution is synonymous to large, shallow open high rate algal ponds (HRAP). HRAPs can have individual pond footprints of 300 – 4,000 m^2 (Ben-Amotz, 2008) and treatment periods of 4 – 10 days (Picot et al., 1992). Such attributes are viewed unsuitable for retrofitting to WWTP, particularly in areas of limited land availability. However, development of alternative versions of microalgal reactors that are optimised to overcome the extensive treatment time and associated footprint are being increasingly investigated (e.g. Tuantet et al., 2014; Van Wagenen et al., 2015). Of these, matrix-immobilisation has shown great potential, through enhancing the algal biomass concentration up to 8 times greater than suspended solutions (Chevalier and De la Noue, 1985), coupled with a reduced treatment time of < 3 h demonstrated for the remediation of secondary wastewater effluents to 0.1 $mgP.L^{-1}$ (Chapter 4).

The immobilisation technology is within its infancy in relation to wastewater remediation, and to date is perceived as an expensive alternative when applied at full scale (Zeng et al,

2015). Recent studies have attempted to study and optimise the treatment process through species selection, biomass concentration, lighting and treatment periods whilst observing nutrient remediation performance and bead life span (Chapters 3, 4 and 5). This paper applies these findings to the design of an immobilised microalgal bioreactor (IBR) for wastewater nutrient remediation in order to understand the economic barriers to implementation.

Three alternative, conventional solutions are designed and examined alongside an IBR, to provide context and a reference CAPEX and OPEX for comparison. Critical cost components for the IBR are identified and assessed through a sensitivity analysis to consider the impact of uncertainty and cost evolution on the potential for economic viability against traditional solutions.

6.1.1 Business case scenarios

The study is based on an upgrading scenario of a 2,000 PE WWTW with existing P removal such that the considered wastewater contains an incoming quality of 1 $mg.L^{-1}$ P and 5 $mg.L^{-1}$ ammonia. The base cases incorporate secondary coagulant dosing followed by either a sand filter (Scenario A) or an aerated wetland (AW) (Scenario B) as an additional polish to reach sub 0.1 $mg.L^{-1}$. The scenario has been chosen as a conservative test to emphasis cost criticalities and enable stress testing of the impact of likely cost evolution of the innovative component to provide a conservative assessment of economic plausibility. The findings are then discussed in relation to other factors and application which potentially enhance the overall viability of the IBR solution.

AWs have been shown to nitrify with effluent NH_4-N concentrations of less than 1.0 $mg.L^{-1}$ reported (similar to those observed by immobilised microalgae, (Section 4.3.2)). They are considered an attractive alternative especially as a retrofit where a horizontal flow wetland already exists through the associated reduced capital cost, power consumption and footprint in comparison to alternative tertiary nitrification processes. However, the ability to meet sub 1 $mg.L^{-1}$ phosphorus discharges through chemical dosing with this technology is not known but is anticipated to be plausible.

Scenarios A and B are considered representative of conventional solutions. Scenarios C and D are illustrations of microalgal solutions and include a HRAP as an example of the

most common embodiment of a microalgal reactor, as the alternative to an IBR (Figure 6.1).

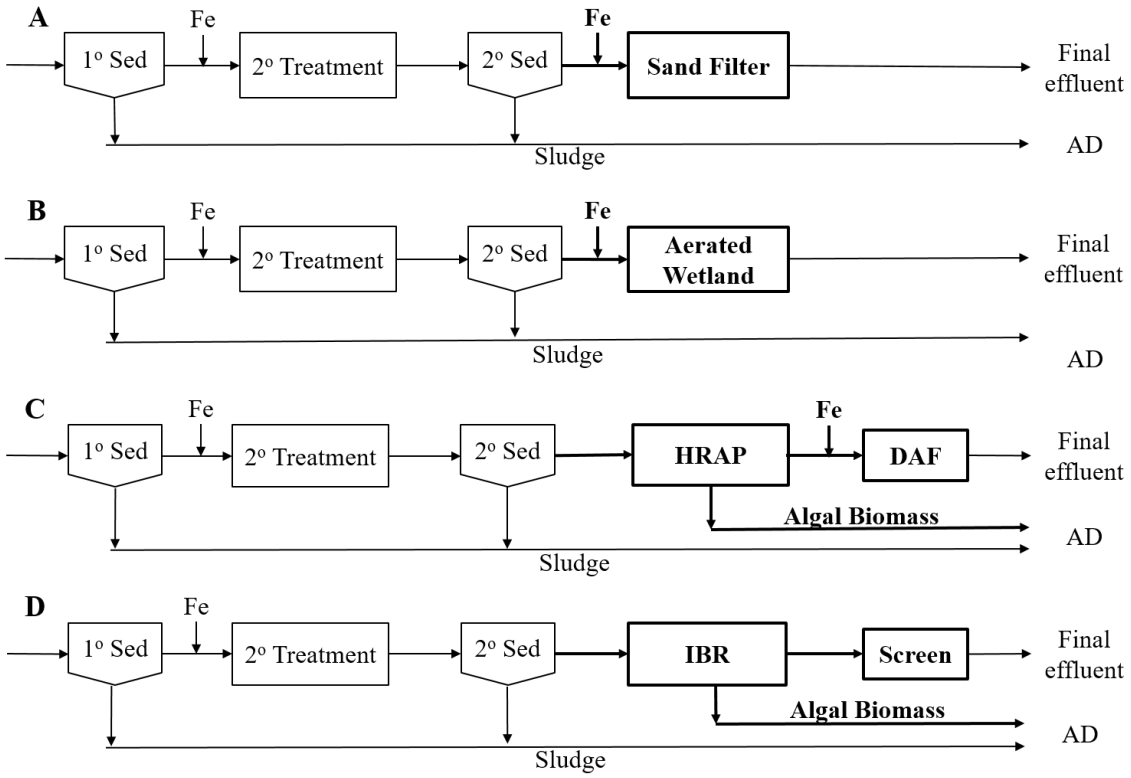

Figure 6.1 Business case scenarios, with the upgraded components emboldened.

An anaerobic digestor (AD) is assumed to be available, with capacity for additional microalgal feedstock with a suitable upfront pre-treatment or advanced digestion for Scenarios C and D (not assumed to be co-located with the IBR or HRAP). Microalgal VS content of 90% is assumed with overall energy conversion of 2.9 $kWh.m^{-3}$ CH_4 considering a combined heat and power (CHP) conversion efficiency of 30 % (Ometto et al., 2013). Biomethane production following enzymatic pre-treatment of 0.48 $m^3CH_4.kg\ VS^{-1}$ is assumed for suspended microalgae (Ometto et al., 2013) and a reduced value of 0.17 $m^3CH_4.kg\ VS^{-1}$ for immobilised microalgae following data from preliminary biomethane potential (BMP) trials.

6.2 Materials and Methods

6.2.1 Design and parameters

All scenarios are validated through design decisions based on the results within the current thesis or based on information within existing literature, with assumptions and omissions for each scenario listed in Table 6.1.

Table 6.1 Summary of assumptions and omissions.

Scenario	Design assumptions and omissions
General	• 200 m^2 available space on site for retrofit of additional assets, further land (brown field) available for purchase if required. • Influent and effluent pipework excluded from all scenarios and assumed included within the factorial method to estimate the CAPEX through the use of an industry specific Lang Factor. • Pump power and associated operational costs estimated using a 50% motor efficiency.
A: Coagulation and sand filtration	• Filter backwash once daily by fluidisation with water and air. • Sludge transport off site, assume capacity available for additional produced sludge for transportation off site with primary sludge with negligible transportation costs. • Asset replacement and refurbishment not included within OPEX estimate.
B: Coagulation and aerated wetland	• Asset replacement and refurbishment not included within OPEX estimate. • No increase in the refurbishment frequency of the wetland.
C: HRAP	• Anaerobic digestor available with capacity for microalgal feedstock with a suitable upfront pre-treatment. • Biomass transport off site, assume capacity available for additional produced biomass for transportation off site with primary sludge, with negligible transportation costs. • Asset replacement and refurbishment not included within OPEX estimate.
D: IBR	• Anaerobic digestor available with capacity for microalgal feedstock with a suitable upfront pre-treatment. • Operation cycle includes one day to fill and one day to empty. • Treatment time modelled on bead uptake rate. • pH increase > 9 and associated precipitation of P observed at HRTs >6 h (bead contact time >2 h) (Section 4.3.1), no dilution required nor design requirement for collection of P precipitate at HRTs <6 h.

The energy balance provided for each scenario relates to the net energy demand (electricity) of the retrofitted assets for the remediation of P to 0.1 $mg.L^{-1}$ including any ancillary equipment for the harvesting of microalgal biomass, and electricity generated by the AD of microalgal biomass post treatment.

6.2.1.1 Scenarios A and B: Conventional solutions design philosophy

Coagulant dosing represents the predominate method of P removal for Scenarios A and B, with downstream removal of P through filtration of the aggregated P particles. Coagulant dose for influent concentrations of 1 $mgP.L^{-1}$ was calculated through a recommended molar dose of 7.42 for the coagulant, ferric chloride ($FeCl_3$) to P for a 90 – 99% removal (Hauduc et al., 2015); with the required dose assisting in the calculation of the required dosing pump, enabling pump sizing and costing. [Design calculations can be located in supplementary information, Appendix E.2.1].

Scenario A represents a continuously mono media sand filter (e.g. Astrasand™, Dynasand™), sized through standard design specifications. Design criteria, operational parameters and quoted energy requirements were taken from the literature (Table 6.2) enabling determination of required pump power for daily operation. [Design calculations can be located in SI, Appendix E.2.2].

Scenario B was designed through an operational design standard of 0.5 $m^2.PE^{-1}$ for tertiary treatment using an aerated horizontal flow wetland (Butterworth et al., 2013) and energy demand cited within the literature (Table 6.2). Design criteria are taken from the literature with design calculations provided in SI, Appendix E.2.3.

Table 6.2 Main design parameters and assumptions for Scenarios A and B.

Design parameter	Value	Unit	Notes	Reference
Inflow	360	$m^3.d^{-1}$	Based on 2,000 PE	
A and B: Coagulant				
Fe:P	7.42		Molar ratio	(Hauduc et al., 2015)
$FeCl_3$ strength	36	%		
A: Sand filter				
Filter feed pump	0.02	$kWh.m^{-3}$		(Cao, 2011)
Filtration	0.01	$kWh.m^{-3}$		(Cao, 2011)
B: Aerated wetland				
Footprint	0.5	$m^2.PE^{-1}$	Based on aerated horizontal flow constructed wetland for tertiary nitrification	(Butterworth et al., 2013)
Energy demand	0.49	$kWh.m^{-3}$		(Austin and Nivala, 2009)

6.2.1.2 Scenario C: HRAP design philosophy

The design parameters for Scenario C (Table 6.3) are based on a simplistic design of excavating earth and covering with a pond liner (Demirbas and Demirbas, 2010). Surrounding walls to contain the pond and provide a barrier to debris are constructed from concrete (also covered by the pond liner), with an internal 'island' wall which assists with the ringed channel structure necessary for mixing. Design criteria for a HRAP are taken from the literature with design calculations provided in SI, Appendix E.2.4.

A maximum hydraulic retention time (HRT) of 10 days is selected through previous calculations which determined a HRT of 5 – 10 days necessary for a suspended mono culture of *S.obliquus* at concentrations seen within a HRAP for the effective remediation of P (Whitton et al., 2016), with the maximum HRT selected to compensate for the seasonal variability of light and temperature (García et al., 2000).

Table 6.3 Main design parameters and assumptions for a HRAP.

Design parameter	Value	Unit	Notes	Reference
Inflow	360	$m^3.d^{-1}$	Based on 2,000 PE	
HRT	10	d	Range 4 – 10 days.	(Picot et al., 1992; García et al., 2000; Whitton et al., 2016)
Individual pond surface area	1,500	m^2	Optimal size area 300 – 4,000 m^2	(Ben-Amotz, 2008; Demirbas and Demirbas, 2010; Jonker and Faaij, 2013)
No. of ponds	8		Calculated	
Total reactor footprint	1.2	ha	Assuming 10 day retention time and 0.3 m depth (0.2 – 0.6 m recommended)	(Borowitzka, 1999; Picot et al., 2009; Norsker et al., 2011)
External pond wall height	0.3	m		Assumption
Island wall height	0.6	m	Pond base to level with external walls	Assumption
Wall thickness	0.3	m		Assumption
Paddle wheel per pond	1		One paddle per 1,500 m^2 pond	(Ben-Amotz, 2008)
Pond length	100	m	Optimal length, 10 – 300 m	(Ben-Amotz, 2008)
Channel width	7.5	m	Optimal width, 1 – 20 m	(Ben-Amotz, 2008)
Laminar flow velocity	30	$cm.sec^{-1}$	Optimal mixing for high productivity	(Richmond, 2004; Ben-Amotz, 2008)
Manning's n	0.012		Smooth plastic on granular earth	(Richmond, 2004)
Paddle wheel efficiency	17	%	Average value, paddle wheels operating over a flat bottom	(Richmond, 2004)
Biomass concentration	0.2	$g(DW).L^{-1}$	Estimated value reported in the literature.	(Craggs et al., 2012)
Harvesting rate	10	%	Assumption ($360m^3.d^{-1}$ removed)	(Rogers et al., 2014)
Biomass removed	7.0	$kg(DW).d^{-1}$	Calculated	

Harvesting (removal) of the suspended microalgae from the treated effluent is accomplished through coagulant dosing to form flocs of the microalgal biomass prior to removal via dissolved air flotation (DAF). DAF is the most commonly applied technology for flotation (Ometto, 2014) and takes advantage of the natural tendency of microalgae to

float (Larsdotter, 2006). Design decisions for suspended biomass recovery were taken from the literature (Table 6.4).

Table 6.4 Main design parameters and assumptions for HRAP biomass recovery.

Design parameter	Value	Unit	Notes	Reference
Coagulant demand	52	$mgFe.L^{-1}$	Based on maximum of 0.15 $g.L^{-1}$ of $FeCl_3$ for flocculation of various microalgal species.	(Chen et al., 2013; Laamanen et al., 2016)
Removal efficiency	90-98	%	Based on 0.15 $g.L^{-1}$ of $FeCl_3$ when harvesting suspended *S.obliquus* grown within a HRAP.	(Chen et al., 2013)
DAF energy demand	0.5	$kWh.m^{-3}$	Conventional energy usages range between 0.2 and 0.5 $kWh.m^{-3}$ of treated water	(Molina Grima et al., 2003)

6.2.1.3 Scenario D: IBR design philosophy

The IBR is based on a modular design (Figure 6.2a) with feed introduced into the base of the reactor creating bead fluidisation. Effluent is recirculated to maintain the chosen HRT, with treated effluent removed through an overflow and passed through a screen to assure the removal of any beads which may escape the module. Lighting is provided by an internal grid of light emitting diodes (LEDs) which cover the depth of the bead bed including the additional bed height through fluidisation (up to 30 % bed expansion at maximum fluidisation velocity), [SI, Appendix E.2.5] (Figure 6.2b). LEDs are the preferred option for illumination through the extended lifetime of the bulbs (≤100,000 h, equivalent to 11.4 years at 24 $h.d^{-1}$), the range of available intensities and wavelengths and reduction in energy consumption (Wang et al., 2007; Yeh and Chung, 2009; Ibrahim et al., 2014) of 50% in comparison to conventional artificial light sources (Chen et al., 2011). As such LEDS are considered more economical in the illumination of microalgae (Wang et al., 2007).

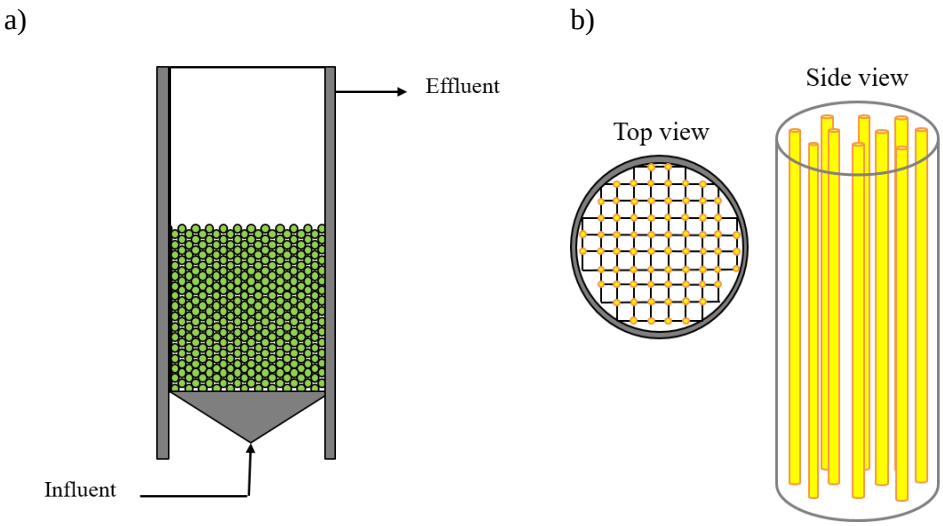

Figure 6.2 a) Schematic representation of an IBR and b) internal LED arrangement.

The lighting system for the modules are based on findings within the current thesis (Chapter 5). Lighting grids consist of white LED bulbs with an intensity of 300 – 400 $\mu mol.m^{-2}.s^{-1}$ and a bulb spacing of 32 mm enabling beads to remain within a critical PFD (photo flux density) band of 50 to 200 $\mu mol.m^{-2}.s^{-1}$ (Section 5.3.2). Each bulb has a standard viewing angle of 120^{o} with 3 bulbs configured back to back to back enabling illumination of a volume of $1.37x10^{-4}$ m^3. The total bead bed volume (including fluidisation) was then divided by the illumination volume of the LED bulb configuration to calculate the number of LED bulbs required per module for the lighting grid system (Figure 6.2b).

The design philosophy of the IBR is adapted from that used for upflow anaerobic sludge blanket (UASB) (Metcalf & Eddy et al., 2003) incorporating the findings from the current thesis (Table 6.5; SI, Appendix E.2.5).

Table 6.5 Key design parameters and assumptions for an IBR.

Design parameter	Value	Unit	Notes	Reference
Inflow	360	$m^3.d^{-1}$	Based on 2,000 PE	
Bead concentration	<40	$Beads.mL^{-1}$	Based on beads with 3 mm diameter and 0.025 mL volume (equivalent bead:wastewater 1:1 v/v)	(Abdel Hameed, 2007; Whitton et al., 2016, Section 3.3.3)
Min. fluidisation velocity	2.64	$m.h^{-1}$	Calculated	
Bed expansion	30	%		
Individual reactor height	3	m		
Individual reactor diameter	<5	m	Assumption	
Light wavelength	400 - 700	nm	White light, based on results within current thesis	(Section 5.3.1, 5.3.2 and 5.5)
Light intensity	300 - 400	$\mu mol.m^{-2}.s^{-1}$	Based on results within current thesis for efficient light utilisation.	(Section 5.3.2)
Light regime	24	$h.d^{-1}$	Constant light, no flashing nor photoperiods - based on results within current thesis	(Section 5.3.3)
LED light spacing	32	mm	Photoactive volume bed depth delivering a minimum of 50 $\mu mol.m^{-2}.s^{-1}$ considering a packed bed	(Section 5.3.2)
LED bulb viewing angle	120	degrees	Standard viewing angle for LED bulbs	
Bulb lifetime	100k	h		(Ibrahim et al., 2014)

The modular reactors are designed on the total P loading and bead uptake rate for immobilised *S.obliquus*, with a reasonable relationship ($r^2 = 0.89$) observed between bead uptake rate and bead batch life (Section 4.3.3, SI Figure A.5); described through Equation 6-1 where y = bead uptake rate ($\mu g.bead^{-1}.d^{-1}$) and x = batch run time (d). This relationship is modelled on experimental studies where nutrient remediation trials were halted through deterioration in performance, and does not include those trials where bead degradation was the cause of the trials being terminated (Section 4.3.3).

$$y = Ln\left(\frac{x}{45.433}\right) \times \left(\frac{1}{-3.196}\right)$$ Equation 6-1

Equation 6-1 enabled calculation of the required bead concentration (total number of beads) and associated bead volume (m^3) assuming a fix bead diameter of 3 mm as observed during laboratory production (Section 4.2.2 and 5.2.1). A total reactor height of 3 m was selected through UASB design guideline recommendation of no greater than 5 – 7 m (Metcalf & Eddy et al., 2003).

Removal of the microalgal biomass prior to a deterioration in treatment performance is accomplished through gravity settlement. The beads sink to the bottom of the reactor when the influent flow and recirculation pumps are switched off, thereby eliminating the costs associated in the removal of suspended biomass i.e. coagulant dosing and harvesting technologies with intensive energy requirements. As a back-up, the treated effluent from the module passes a screen of 1 mm to ensure beads are unable to leave the module within the effluent.

The IBR design was required to satisfy a number of design gateways including; a minimum bead contact time of 2 h (equivalent to 6 h HRT as determined in Chapter 4) for optimal remediation performance and prevention of significant pH increases within the treated effluent (Chapter 4), a bead concentration < 40 beads.mL^{-1} (equivalent to a bead:wastewater v/v of 1:1) through practical limitations resulting in the beads sinking and crushing under their own weight (Abdel Hameed, 2007) (Section 3.3.3) and, a modular diameter of < 5 m considered suitable for retrofit at a small WWTP. The final IBR design consists of 3 parallel modules with a treatment period (HRT) of 2 h and a bead batch length of approximately 17 days. Each module is 3 m in height with a diameter of 2.5 m containing 5.2 m^3 beads. Lighting grids within each module consist of 35,683 white LED bulbs with each module covering a footprint of 4.9 m^2 and a total land footprint for all modules of 37.7 m^2 when considering necessary spacing between modules for access and service requirements. [see Appendices E.2.5 for detailed design and cost data].

6.2.2 Economic evaluation

6.2.2.1 Capital cost estimates

The capital costs (CAPEX) were calculated in British Pound Sterling (£) and converted to 2015 prices using the Consumer Price Index (CPI) (Office for National Statistics, 2016) overall index for the period of 2002 – 2015 (SI, Appendix E.3) with estimated purchase costs for major equipment items obtained from a combination of literature and water company data (Table 6.6).

Table 6.6 Summary of capital cost estimates for major components.

Design parameter	Value	Unit	Notes	Reference
General				
Excavation	3.49	£.m-3	Excavation to a depth of 0.3 m	(ICE, 2010)
Land purchase/take cost (brown field)	42.00	£.m^{-2}		(Department for Communities and Local Governments Resource, 2015)
Land preparation	0.21	£.m^{-2}		(ICE, 2010)
Concrete	93.05	£.m^{-3}		(Langdon, 2008)
Scenario A: Coagulation and sand filter				
Sand filter	1,008,913	£	Based on design flow	(D. Inman, 2016, pers. comm, 2nd February)
Coagulant dosing system	55,379	£	Including storage tank, transfer pumps, metering pumps, piping and valves and facility enclosure	(McGivney and Kawamura, 2008)
Scenario B: Coagulation and aerated wetland				
Installation cost	350	£.PE^{-1}	Assume costs include land preparation and installation of aeration system.	(D. Inman, 2016, pers. comm, 2nd February)
Coagulant dosing system	55,433	£	Including storage tank, transfer pumps, metering pumps, piping and valves and facility enclosure	(McGivney and Kawamura, 2008)

Continued...

Design parameter	Value	Unit	Notes	Reference
Scenario C: HRAP				
Pond liner	4.57	£.m^{-2}	Lake liner, all joints welded, 1 mm thick	(Langdon, 2009)
Paddlewheel	12,076	£	Paddlewheel with 1.74 kW motor.	(Rogers et al., 2014)
Influent/effluent pump	2,767	£		(Loh et al., 2002)
DAF unit	175,046	£	Based on design flow	(D. Inman, 2016, pers. comm, 2nd February)
Scenario D: IBR				
Carbon steel tank	1,136	£.m^{-3}		(Sinnott, 2005)
LED lighting system	2.98	£.bulb^{-1}	Including all ancillary equipment.	(iXscient Ltd, 2014, pers. comm, 22nd October)
Screening unit	94,246	£	Flow rate equiv. 0.25m^3.min^{-1}	(Loh et al., 2002)
Centrifugal pumps	3,874	£		(Loh et al., 2002)

Upon totalling the CAPEX estimates of the purchase cost of the major equipment items for each scenario, the remaining direct and indirect costs were estimated by the factorial method of multiplying the CAPEX estimate by an industry specific Lang Factor (LF) of 2.5 (D. Inman, 2014, pers. comm, 2nd May). The factorial method was utilised for processes designed from first principles (i.e. coagulant dosing system, HRAP and IBR), but not applied to those scenarios were a fixed CAPEX was known for the construction of an asset treating a similar PE. Preliminary estimates based on this method offer a ±30% accuracy which are suitable for early feasibility studies to inform initial decisions between alternative technologies (Sinnott, 2005).

6.2.2.2 Operational cost estimates

The operational costs (OPEX) were calculated in British Pound Sterling (£) and converted to 2015 using the CPI as previously described (SI, Appendix E.3) with an OPEX cost estimates obtained from a combination of literature and water company data (Table 6.7).

Table 6.7 Summary of operational cost estimates and energy consumption.

Parameter	Value	Unit	Notes	Reference
General				
Electricity	0.085	£.kWh^{-1}	Industrial user cost	(D. Inman, 2014, pers. comm, 2nd May)
Scenario A and B: Coagulation				
$FeCl_3$	290	£.ton^{-1}	Industrial bulk purchase price	(M. Pidou, 2016, pers. comm, 27th January)

6.2.2.3 Sensitivity analysis

Further investigation was undertaken to establish the sensitivity of the CAPEX and OPEX to the identified major components contributing to the overall cost of an IBR. Sensitivity analysis was completed by varying one parameter cost by 100% in increments of 5%, whilst fixing the cost of all the other parameters contributing to CAPEX and OPEX. The parameters selected for sensitivity analysis include LED bulb cost, screening unit cost, bead manufacture cost and LED bulb power.

6.2.2.4 Whole life cost estimate

The WLC period is defined as 40 years and calculated using Equation 6-2 assuming a discount rate of approximately 7%. The WLC comprises the initial CAPEX and OPEX associated to ongoing operational and maintenance expenditures including asset replacement and refurbishment costs over the whole life period (Anglian Water, 2010).

$$WLC = CAPEX + (OPEX \times 14) \quad \text{Equation 6-2}$$

6.3 Results and Discussion

6.3.1 Capital cost estimate

Initial CAPEX estimates determine the installation of an IBR to be £1.2M compared to £2.3M for the HRAP, representing a 52% reduction in CAPEX. Comparison to the CAPEX estimates for the non-algae based solutions (Scenarios A and B) revealed no significant difference between options within the ± 30% variation at £1.3M and £1.0M respectively (Figure 6.3). The increased CAPEX of the HRAP was related to the 1.2 ha footprint of the option with land purchase contributing 54.3% of the CAPEX (Figure 6.4a). However, if land is available for use and excluded from the estimate, the DAF becomes the major costing item (representing 41.9% of the amended CAPEX estimate)

with a reduced total CAPEX of £0.8M then making it a lower CAPEX option than all Scenarios.

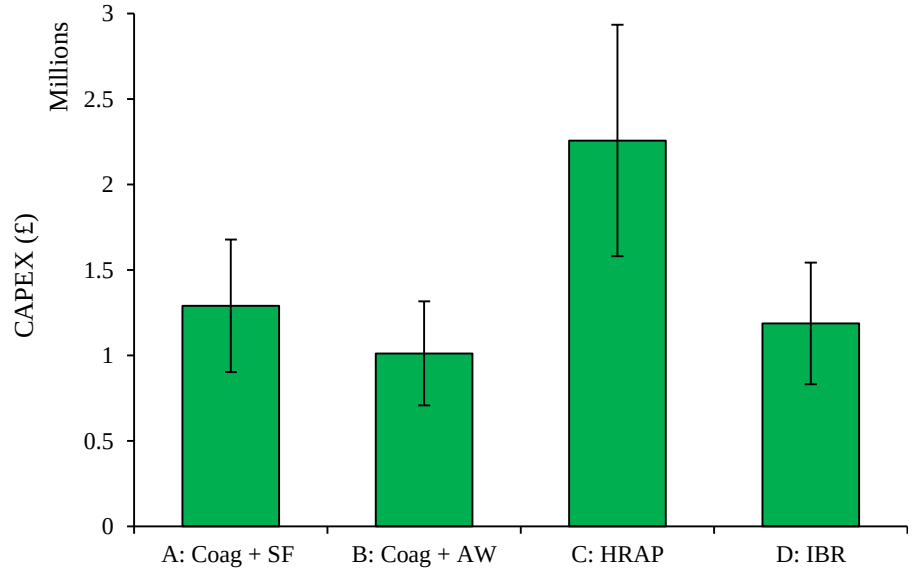

Figure 6.3 CAPEX estimates for Scenarios A – D (± 30% variation).

The major cost critical components of the IBR were identified as the LEDs and the screen (Figure 6.4b) contributing 86.7% of the total CAPEX. Sensitivity analysis revealed that the CAPEX reduced from its most conservative estimate of £1.2M to £1.14M, £1.07M and £1.0M with a reduction in the cost of the screen of 20%, 50% and 80% respectively. Much greater impact is seen through the reduction in the estimate of the bulb price which reveals a reduction in total CAPEX to £1.02M, £0.78M and £0.58M for equivalent reduction in bulb price whilst using the highest estimate for the screen (Figure 6.5).

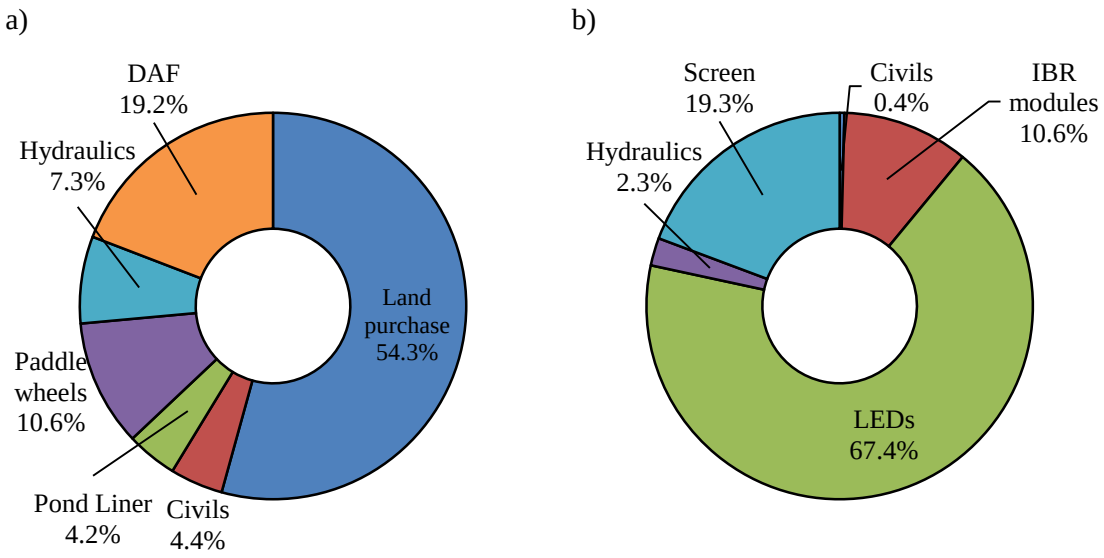

Figure 6.4 Contribution of major equipment to the CAPEX for a) Scenario C (HRAP) and b) Scenario D (IBR).

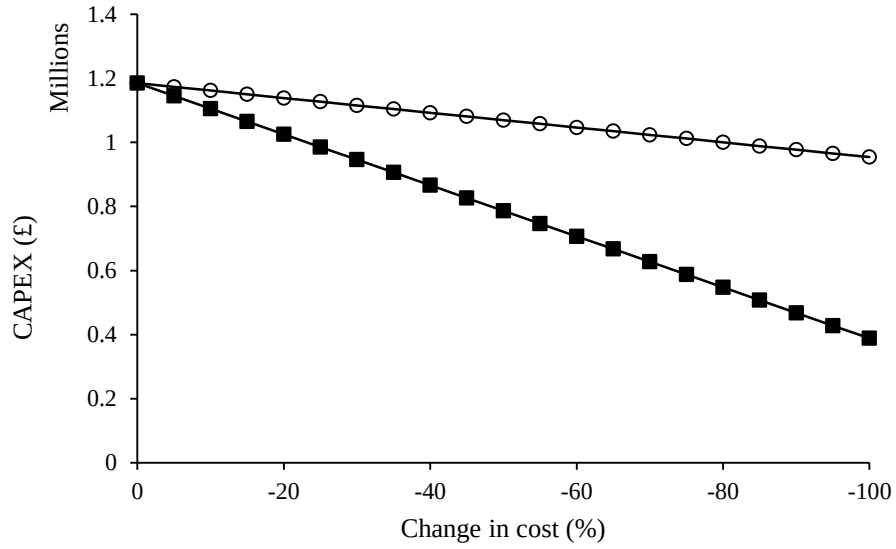

Figure 6.5 Change in CAPEX estimate with technology development. (■) £.bulb^{-1} and (○) screen cost (£).

The reduction in CAPEX due to the bulb cost can be achieved through either a reduction in the unit cost of the bulb or improvements in the lighting design. Consideration of the former through analysis of the literature suggests that a conservative estimate of the reduction in bulb costs can be predicted to be between 30 and 60% over the next 10 years. For instance, the cost of household LED bulbs has reduced by between 22 – 44% between

2012 and mid-2014 (Gerke et al., 2014). Further, cost predictions beyond 2020 for white light LEDs suggest that operating cost would be considered the dominant factor during the decision for purchase and use (Haitz and Tsao, 2011). Whilst in an earlier stage of development UV LED have seen a similar cost reduction profile, with cost reductions of 98% predicted between 2015 – 2020 resulting in a bulb price of £0.05 – 0.07 (Autin et al., 2013; Ibrahim et al., 2014). Overall changes in bulb efficiencies and prices have been shown to follow 'Haitz's Law' (Ibrahim et al., 2014) providing confidence in the proposed reductions.

Further improvements in design of lighting arrays can be expected through the experiences gained in the initial demonstration installations such that a further 10% reduction in equivalent bulb cost is predicted over the next 10 years. Overall a 50% reduction in bulb price is considered a plausible yet cautious expectation for predicting future costs.

Upon the future cost reduction in white LEDs, the screening unit becomes a more significant cost item representing 25.3% of the total CAPEX. However, the capital cost of the screening unit has the potential to reduce through innovation and technology development. Cost reductions for such units, including non-modular units and plants, are known to decrease slowly through analysis of experience curves (Neij, 2008) and as such a 10% cost reduction per decade has been assumed. Therefore, the CAPEX cost of an IBR in 2025 providing technology development and associated price reduction would be £0.77M equivalent to a 41.7% reduction. At this cost the IBR shows the lowest CAPEX at 53.8, 70.0 and 30.4% of Scenarios A, B and C respectively.

6.3.2 Operational cost estimate

No commercial supply chain for immobilised microalgae currently exists and as such the unit costs of beads represents the major uncertainty in the OPEX calculation of the IBR. To understand the significance of this cost item analysis on the impact of changing bead costs on the overall OPEX of the IBR was conducted (Figure 6.6). A reduction in OPEX from £44k.year^{-1} to £19.5k.year^{-1} was observed as the bead cost reduced from £1 per 10^6 beads^{-1} to £0.01 per 10^6 beads^{-1} (Figure 6.6). The residual OPEX represents costs associated with energy supply for lighting and pumps and reveals the OPEX of the IBR

to be greater than the other options which were estimated to have OPEX costs of £4.5k, £9.7k and £8.1k for Scenarios A, B and C respectively (Figure 6.6).

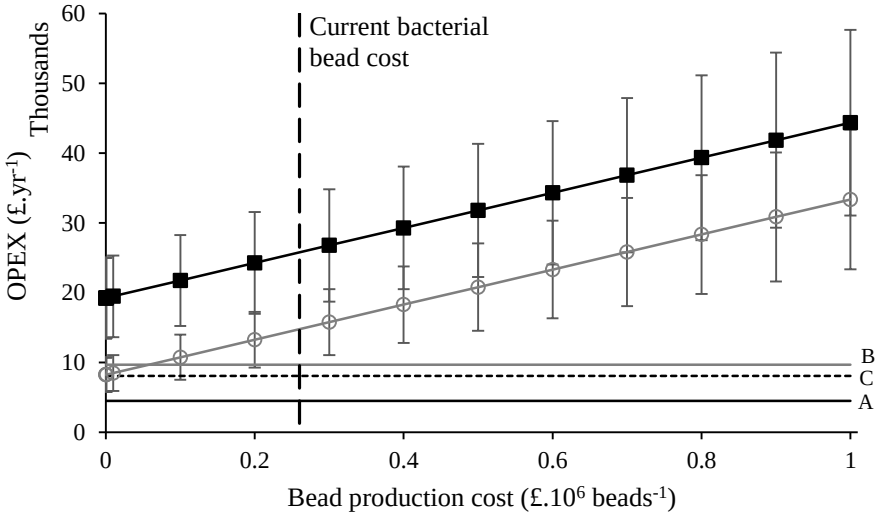

Figure 6.6 Change in OPEX estimate with bead production cost in comparison to OPEX costs for Scenarios A, B and C (± 30% variation). (■) bead production cost only and (●) bead production cost including income through energy recovery from AD.

However, this initial cost estimate is based on a bead batch length of approximately 17 days from trials within this thesis using a sodium alginate resin (Chapter 4). Further development can realistically be expected to extend bead life with up to a 34 day cycle time considered plausible. Extending the batch life, reduces the annual number of bead replacements, reducing the annual bead cost by 33.3%.

A key benefit in the use of microalgae is the ability to recover energy through digestion of the biomass which represents a cost recovery component of OPEX. Estimates of the energy recovery suggests that between £1.24 per 10^6 beads^{-1} to £1.54 per 10^6 beads^{-1} is possible. Inclusion of this recovered income significantly impacts the overall OPEX such that at a bead cost of £0.01 per 10^6 beads^{-1} reduces the OPEX to an estimate 12.4% less than Scenario B and 4.7% greater than Scenarios C (Figure 6.6).

Consideration of whether such a production cost could be likely in the future was established through comparison to prices gathered from a commercial supplier of immobilised microorganisms (bacteria, enzymes and yeast) (Table 6.8). Commercial production costs, predict an equivalent bead production cost of approximately £0.26 per 10^6 beads^{-1} could be possible (Table 6.8), suggesting further cost reductions would be

required to enable the IBR process to be comparable to the alternative technologies. Whilst exact translation to immobilised microalgae is difficult, analysis indicates that it is plausible for a supply chain to be available for immobilised microalgae in the future.

Table 6.8 Manufacturer bead production costs

Manufacturer	Immobilised organism	£.kg^{-1}	Equivalent cost. (£.10^{6} beads^{-1})[a]	Reference
Lentikat's Biotechnologies	Bacteria	10.58	0.26	Lentikat's Biotechnologies (2013)
	Enzymes	10.40	0.26	
	Yeast	10.56	0.26	

[a] Calculations located in SI, Appendix E.4.

Furthermore as previously discussed, research and development into LED technology is facilitating improved performance (Steele, 2007; Haitz and Tsao, 2011; Chang et al., 2012), with a trend known as 'Haitz's Law' observed whereby the total output per Watt (W) decreases by a factor of 20 every decade (Haitz et al., 1999). This trend has recently been re-evaluated by Haitz and Tsao (2011) and still found to be applicable when predicting trends within current LED development. As such, advances within LEDs should be considered when predicting the future economic viability of an IBR. Providing bead production costs of £0.26 per 10^{6} beads^{-1}, operational costs for LEDs would become the dominant component, contributing up to 67.3% of the total OPEX.

Providing such technological advances are achieved within the LED industry as predicted by Haitz's Law, the bulb power consumption is predicted to reduce from the present 0.24 W.bulb^{-1} to 0.012 W.bulb^{-1} whilst providing the same output by 2025. The impact of the reduction in bulb power would enable an OPEX cost 77.3% and 92.6% of Scenarios B and C respectively, but 66.7% greater than Scenario A (Figure 6.7). Upon consideration of energy recovery, a 0.06W bulb would enable approximate cost neutrality at the highest potential energy recovery (Figure 6.7). Subsequent reductions of 10% and 20% in energy recovery results in a comparative OPEX for bulb powers of 0.04W and 0.02W respectively with these bulb powers predicted within the next 8 to 9 years.

Bulb powers less than 0.05W combined with maximum energy generation forecast a net income of up to £3.4k per annum at the predicted bulb power in 2025 of 0.012W (Figure 6.7).

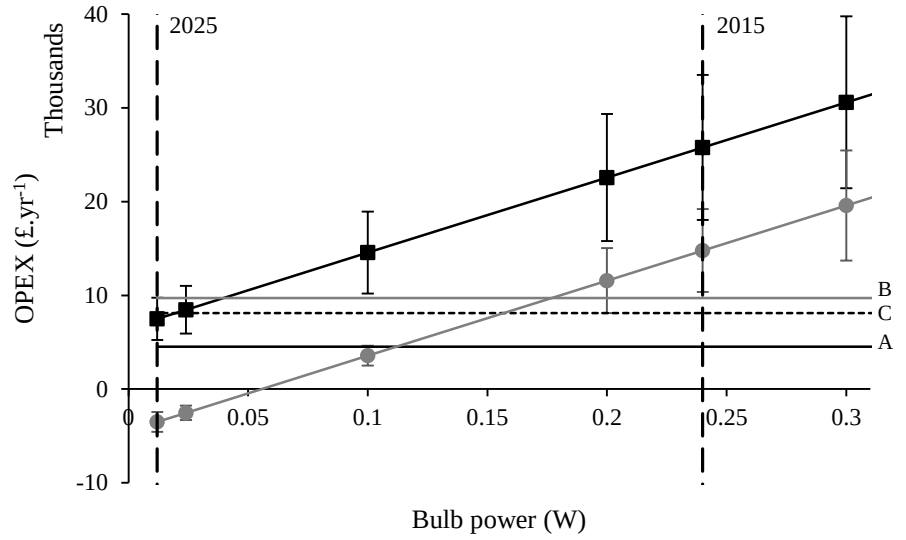

Figure 6.7 Change in OPEX estimate with bulb power consumption in comparison to OPEX costs for Scenarios A, B and C (± 30% variation). (■) bulb power consumption only and (●) bulb power consumption including income through energy recovery from AD.

The OPEX cost estimate within the next decade for an IBR, when considering the discussed technological developments in bead production and LED bulb power considering no energy recovery, is found to be 40.7% greater than Scenario A and 78.4% and 90% the cost estimate of Scenarios B and C respectively (Figure 6.8). When including energy generation, an IBR is found to generate an annual income of approximately £3.4k (Figure 6.8) and would be considered preferable over the alternative options.

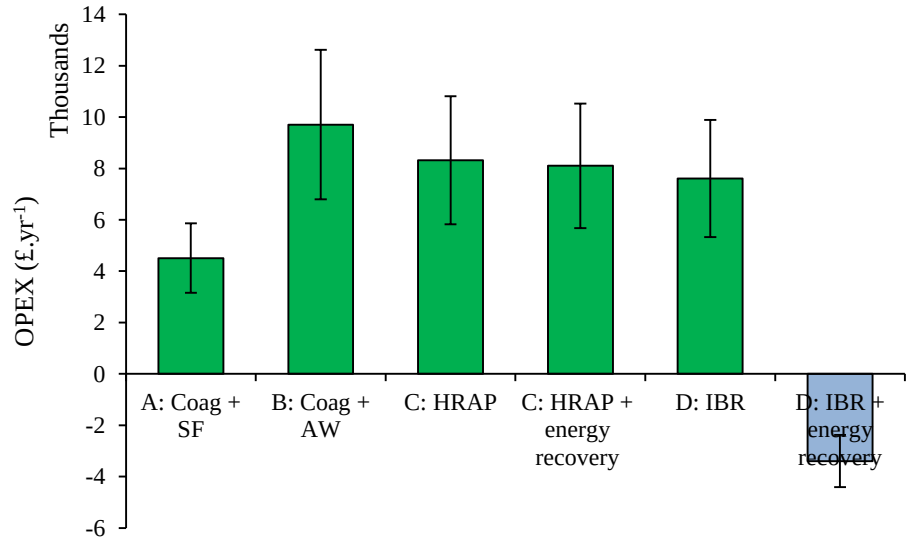

Figure 6.8 OPEX estimates for Scenarios A – D considering 2025 cost estimate for the IBR (± 30% variation).

The major cost component of the IBR OPEX (following the predicted cost reductions in 2025) was identified as the beads (Figure 6.9) contributing to 85.3% of the total OPEX, confirming the significance of the importance of future development to reduce production costs and/or extended bead life.

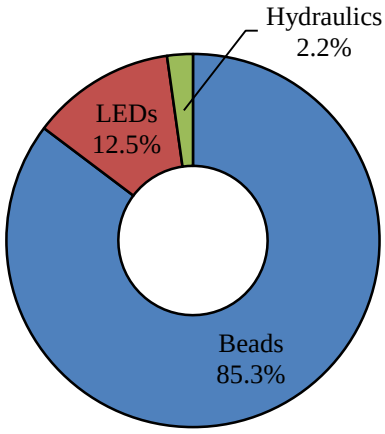

Figure 6.9 Contribution of major equipment to the OPEX for Scenario D (IBR).

6.3.3 Whole life cost

Predictions of cost reductions of major components, namely LED bulbs, screening unit and bulb power consumption, for an IBR estimates an approximate 43.7% and 38.5% reduction in the WLC for scenarios with and without energy recovery to £0.9M and £0.8M respectively over the next decade (Table 6.9). Present day WLC estimates with energy recovery for an IBR are comparable to Scenario A (approximately £1.3M), and 30.0% greater and 41.0% less than Scenarios B and C respectively. Over the next 10 years, the WLC of an IBR with energy recovery becomes even more favourable and predicted to be 61.5%, 80.0% and 36.4% of that of Scenarios A, B and C respectively (Table 6.9).

Table 6.9 Design and economic summary of evaluated solutions for wastewater P remediation.

Scenario	Footprint (m^2)	Energy demand ($MWh.yr^{-1}$)	OPEX (£k)	CAPEX (£M)	WLC (£M)
A: Coagulation and sand filtration	3.0	4.0	4.5	1.2	1.3
B: Coagulation and aerated wetland	1,000	65.1	9.7	0.9	1.0
C: HRAP with energy recovery	12,004	88.6	8.1	2.1	2.2
D: IBR **(2015)**	37.7	227.1	25.8	1.2	1.6
D: IBR with energy recovery **(2015)**	37.7	97.7	14.8	1.2	1.3
D: IBR **(2025)**	37.7	13.3	7.6	0.8	0.9
D: IBR with energy recovery **(2025)**	37.7	-116.1	-3.4	0.8	0.8

Negative value represents income.

The IBR solution offers additional added value in comparison to the conventional solutions which have not been costed within this analysis. For instance, immobilised microalgae have demonstrated enhanced total nitrogen (TN) remediation, with 98.5% remediation of NH_4-N (ammonium) and 51.7% remediation of NO_3-N (nitrate) from secondary effluent concentrations of 4.2 and 20.3 $mg.L^{-1}$ NH_4-N and NO_3-N respectively (Chapter 4.3). Similar TN remediation performance is not possible through the use of a sand filter with only nitrification achievable through an aerated wetland. As such, the application of an IBR may enable further cost reductions for nitrification and denitrification strategies upstream enabling an improved WLC. Furthermore, the appropriateness of the other options to meet a 0.1 $mg.L^{-1}$ standard is uncertain and may require alternative technologies to be utilised such as membrane or multistage depth filter (A. Brookes and P. Vale, 2016, pers. comm, 28th January) significantly enhancing the economic competitiveness of the IBR.

Additionally, the basis of this cost estimate has focused on the remediation of P from a starting concentration of 1 $mg.L^{-1}$. This scenario was selected in order to stress test the selected scenarios under a worst case scenario and identify those components which

would heavily effect the result of the economic analysis. When applying the 2025 IBR cost estimates to an increased P load of 10 $mg.L^{-1}$, bead production (representing the chemical cost of an IBR) is greater than the cost of applying a greater coagulant dose required for the additional P load (Table 6.10). However upon consideration of energy recovery through the additional microalgal biomass, the chemical cost associated to the manufacture of the beads is less than that of the application of coagulant. This is demonstrated for P loads ranging from 1 to 10 $mg.L^{-1}$ (Table 6.10) with an IBR protecting against future increases in the cost of coagulant (Keeley et al., 2014) and closing the resource loop within the circular economy.

Table 6.10 Coagulant dosing vs bead cost plus energy recovery for differing P loads considering chemical costs.

P Load ($mg.L^{-1}$)	**10**	**1**
Coagulant cost (£$k.yr^{-1}$)	42	4
Bead cost (£$k.yr^{-1}$)	73	7
Difference (£$k.yr^{-1}$)	**31**	**3**
Bead energy generation (£$k.yr^{-1}$)	-125	-11
Total (£$k.yr^{-1}$)	**-94**	**-8**

Negative value represents income.

Overall, a cautiously optimistic prediction of the economic viability of the IBR can be provided. Whilst there remains high levels of uncertainty with respect to a number of key cost components the above analysis confirms the appropriateness of further development and larger scale demonstration to reduce such uncertainty and enrich development to minimise cost. For instance, replacement costs have been excluded from the analysis but LED replacement is likely to be a critical factor due to its overall impact on CAPEX. However use of intermittent lighting and reactor operations that enable greater light penetration will reduce total costs. To illustrate, the lighting system was original designed for a packed bed column. Switching to a fluidised system is likely to extend light penetration by at least 80% which is sufficient to offset the replacement cost of the bulbs over the entire time frame of the cost assessment.

6.4 Conclusions

- Present day application of an IBR solution would be considered economically viable in comparison to coagulation and sand filtration for the remediation of wastewater P to 0.1 $mg.L^{-1}$ providing a commercial supply chain for immobilised microalgae and full energy recovery.
- Future commercial supply chains for immobilised microalgae are plausible in view of the available market for other immobilised microorganisms.
- Predicted cost reductions in major components through technology development (i.e. bead and LED cost and LED power consumption), predicts an IBR to be economically viable in comparison to conventional solutions within the next decade.
- Application of an IBR in 2025 for the treatment of higher P concentrations (10 $mg.L^{-1}$) would be economically preferable in comparison to increased coagulant dosing when considering energy recovery, providing protection against future increase in coagulant cost.

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Chapter 7. Implications of the Work: Overall Perspective on the Appropriateness of Algae Reactors for Wastewater Treatment in the UK

The overall aim of the research was to understand and critically evaluate the technical and economic challenges associated with implementation of technologies utilising freshwater microalgae as a nutrient polishing process within wastewater treatment. Through the course of this thesis, the less-favourable characteristics associated to microalgal solutions which limit widespread implementation are challenged through the concept of a new reactor design. The following is a discussion of the concerns raised by the industrial sponsors of this research concerning the implementation of a microalgal solution and the findings from the current thesis which resolve these concerns through the use of an immobilised solution (IBR).

7.1 How does an IBR overcome the implementation challenges associated with HRAPs?

The most common embodiment of a microalgal solution for wastewater treatment is a high rate algal pond (HRAP) (Christenson and Sims, 2011). As a suspended microalgal solution of dilute biomass concentration, a HRAP is associated to large land footprints and extensive treatment periods (Table 7.1) viewed impractical for retrofit at most wastewater treatment works (WWTW). Immobilisation enables concentration of biomass within a reduced overall volume, for instance 10^6 cells.bead^{-1} within a bead volume of 0.025 mL (Chapters 3, 4 and 5), offering a 40 fold increase in biomass concentration in comparison to a suspended solution with typical cell concentration (observed during laboratory cultivation for *Scenedesmus obliquus*) equivalent to 10^6 cells.mL^{-1}. Land footprints associated to IBR implementation of approximately 40 m^2 are possible equivalent to 0.02 m^2.PE^{-1} in comparison to 6 m^2.PE^{-1} (Table 7.1).

Table 7.1 Comparison of the main attributes of a HRAP and IBR found through this thesis.

	HRAP	IBR
Footprint	Full scale demonstration plants with individual pond footprints of 1.25 ha (Craggs et al., 2012; Sutherland et al., 2014), equivalent to 6 $m^2.PE^{-1}$ (Chapter 6)	Land footprints of approximately 40 m^2, equivalent to 0.02 $m^2.PE^{-1}$ (Chapter 6).
HRT	Treatment periods from 7 – 10 days (Picot et al., 1992).	Treatment times from 2 h (Chapter 4 and 6).
Biomass: species and concentration	Seasonally variable biomass concentrations of up to 0.2 $gDW.L^{-1}$, consisting of a symbiotic and changing community of microalgae and bacteria (Craggs et al., 2012; García et al., 2000; Park and Craggs, 2010; Powers and Baliga, 2010).	Increased biomass concentration (40 fold) within a reduced overall volume (Thesis, Chapters 3, 4, 5). Species and biomass concentration control through $beads.mL^{-1}$ and $cells.bead^{-1}$ (Chapter 3).
Lighting requirements	Solar photosynthetic efficiency of approximately 1.5% (Norsker et al., 2011), light variability throughout the year.	High remediation efficacy under LED lighting with the lighting regime for optimal performance identified enabling year round performance (Chapter 5).
Harvesting biomass post-treatment	Harvesting suspended biomass is energy intensive, representing 20 - 30% of the total production costs (Liu and Vyverman, 2015; Zeng et al., 2015).	Beads recovered by gravity settlement, minimal energy or chemical costs.
Economic viability	Inexpensive to install and operate providing land is available. If land purchase is necessary, CAPEX costs are comparable to intensive alternative solutions with WLC £2.2M (Chapter 6).	Predicted cost reductions in major cost components of an IBR estimate a comparable WLC (£0.8M) to conventional solutions utilising coagulation within the next 10 years (Chapter 6).

The intensification of microalgal biomass through immobilisation also significantly reduces treatment times to as little as 2 h (Chapter 4), representing a fraction of the time associated to HRAPs (Table 7.1), by the combined processes of direct and indirect remediation (Chapter 2 and 4).

Indirect remediation of P through amorphous calcium phosphate precipitate was found to contribute between 33.3 – 88.5% during lab scale trials (Section 4.3.1), similar to the performance demonstrated by suspended systems of 16 – 63% (Larsdotter et al., 2007). Conventionally the removal of this precipitate would be achieved through the addition of coagulant to further aggregate the particles in to larger flocs for subsequent removal through settlement. This method of recovery is unable to separate the algal biomass from the precipitate within a HRAP, with the P resource lost during subsequent downstream processing i.e. pre-treatment and anaerobic digestion. However, an IBR offers an option of recovering and separating this precipitate from the microalgal biomass through the difference in the fluidisation properties and/or size of the P granules in comparison to the microalgal beads. Modification of the IBR design to incorporate features of a process designed to control and collect precipitate, such as the Crystalactor®, would enable recovery of the P resource and further contribute to the circular economy through the cascade of recovered P in either the direct use, or as an intermediate product for fertilisation (Giesen, 1999).

A further benefit of an immobilised system is the ability to control the species within the reactor, thereby enabling a chosen species to dominate. Similar species control within an open HRAP is more challenging with predation and contamination of native microalgal species (Park et al., 2011) resulting in a variable and changeable community (Table 7.1). This changing community may not represent optimal remediation characteristics. Findings from the current thesis find nutrient remediation performance of freshwater species can be correlated to the species' specific internal N:P (nitrogen:phosphate) composition (Chapter 3). Knowledge of a species N:P character enables the selection of an appropriate species for immobilisation, with species characterised by a high internal N and low P (molar ratio > 18), demonstrating a higher degree of remediation efficiency (Chapter 3). Seeding the IBR with a species characterised for enhanced remediation enables consistent performance, unaffected by a changing microalgal community.

Light is recognised as a key design feature for microalgal solutions (Lee and Lee, 2001). As such, seasonal variations in biomass concentrations are observed within HRAPs, with maximum biomass concentrations between May to July, with a 25% reduction for all other months excluding November to February with a further 25% reduction (Craggs et al., 2012; García et al., 2000; Powers and Baliga, 2010). The resulting variability in biomass concentration has impacts on the treatment period and remediation efficacy, with constant illumination necessary for consistent performance.

Whilst employing LEDs within an IBR has shown to initially contribute to a significant proportion on the total costs (67.8% CAPEX) (Chapter 6), bulb requirements are based on experimental light attenuation data based on a packed bed configuration considered a worst case scenario (Chapter 5). A fluidised configuration will enable a greater depth of light penetration, reducing the required number of bulbs and further reducing the costs associated to the LEDs. In addition reducing the overall reactor volume through biomass intensification, reduces the volume which requires irradiation with reduced CAPEX and OPEX expenditures, in comparison to irradiating dilute cultures in larger volumes. The research within the current thesis recommends a light regime proven beneficial for immobilised microalgal productivity and remediation performance, preventing the variability in productivity observed within HRAP (Table 7.1).

Finally, whilst a HRAP is inexpensive to install and operate, the limiting factor for implementation is land. Land purchase was shown to contribute approximately 54.3% of the CAPEX when designed and costed for a 2,000 PE (population equivalent), 47.8% greater than an IBR (Chapter 6). If land is available, the WLC of a HRAP is comparable to an IBR (following development in 2025) at £0.9M and £0.8M respectively. However, limited land availability around the smaller WWTWs suggests the economic viability of an IBR in the next decade to be favourable in comparison to a HRAP, and further supports the implementation of an IBR over a HRAP.

7.2 What are the key implementation challenges of an IBR?

Findings within this thesis challenge the common conceptions associated to the use of microalgae for wastewater nutrient remediation through immobilisation. However, implementation challenges remain before widespread application can be achieved.

Currently, there are no commercial supply chains for the cultivation and immobilisation of microalgae. Supply chains for yeast, bacteria and enzymes exist with immobilised nitrifying bacteria available specifically for the wastewater industry (Lentikat's Biotechnologies, 2013). Given the recent interest in the application of microalgae, a future supply chain is plausible. The un-optimised bead production protocols adopted within the current thesis utilises a natural and costly resin, sodium alginate. An optimised industrial process may determine a more effective methodology using a more cost effective resin, enabling a production cost which supports a profitable supply chain. Accordingly, further development around bead life and supply is a key area for future development (see Chapter 8, Further Work).

Furthermore, owing to effect of NH_4^+ and NO_3^- uptake and the impact on wastewater pH (Section 4.3.2), certain wastewaters will be unsuited for treatment by immobilised microalgae. For example, a wastewater with a high NH_4-N concentration and little NO_3-N will create an acidic environment during treatment (Xin et al., 2010) with the potential to consume alkalinity and the associated buffering capacity. In such cases, the environment will become unfavourable for microalgal growth and prohibit nutrient uptake generating an acidic and untreated effluent. Options for controlling pH can be adopted when there is an unsuitable balance of NH_4-N and NO_3-N, with CO_2 sparging commonly used for suspended solutions (Park and Craggs, 2010) to ensure a neutral pH and remediation through direct mechanisms. As such, initial trials should be conducted with the source water to determine the impact of microalgal treatment.

Providing adequate concentrations of nitrogen species, an increase in pH to a maximum of 11.1 was observed during lab trials (Section 4.3.1). To limit effluent pH to a more acceptable pH $\leq$ 9, a bead contact time of $\leq$ 2 h (equivalent to HRTs of $\leq$ 6 h during lab trials), should be adopted. The peak in pH was observed for only a short period during the initial start-up, prior to returning to the approximate pH of the feed (Section 4.3). As such the IBR modules should be run in series, ensuring the start-up periods characterised by the initial peak in pH do not occur simultaneously for all modules. Treated effluent from the modules can then be blended to compensate for the increased pH from one module, preventing treated effluent pHs unsuitable for discharge.

7.3 When is it suitable to use an IBR over other tertiary P solutions?

Providing challenges to implementation can be overcome, application of an IBR would be viewed more suitable than treatment strategies aligned to a linear economy where resources are un-recovered e.g. coagulation. An IBR protects against the increasing cost of coagulant (Keeley et al., 2014) whilst providing an energy neutral to positive process (Chapter 6) as aspired within the attainment of a circular economy. Further contribution to the circular economy through possible material cascading e.g. calcium phosphate for fertiliser, offers further benefits in comparison to conventional chemical treatment strategies.

Furthermore, an IBR remediates total nitrogen (TN) and is a complete nutrient remediation technology. An IBR therefore offers wider benefits than a technology designed for the sole remediation of P and can be considered in the place of multiple technologies required to further polish P and N residual concentrations.

7.4 References

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Chapter 8. Conclusions and Future Work

8.1 Conclusions

The overall findings of this research demonstrates freshwater microalgae can be considered a viable option for nutrient polishing within wastewater treatment. The specific conclusions in relation to the original objectives are as follows:

Objective 1. To produce a state of the art critical review on microalgal technologies for nutrient remediation to inform the selection of a technology for further research and development.

- Varying bioreactor designs are available including suspended and non-suspended (i.e. immobilised) systems, with sub-categories of either open to the environment or enclosed within photobioreactors (PBR) (Chapter 2).
- A high phosphorus (P) remediation efficiency at HRTs of up to 2 days are demonstrated by immobilised microalgae and PBRs, representing solutions with the greatest biomass concentration (Chapter 2).
- Similar remediation efficacy for ammonium (NH_4-N) is demonstrated by immobilised microalgae and PBRs within HRTs of 2 days (Chapter 2).
- Benefits of immobilisation further include low costing harvesting following treatment through gravity settlement, eliminating chemical and energy costs associated to suspended systems (Chapter 2).
- Increasing demonstration of non-suspended systems will better enable appropriate comparison to be made with high rate algal ponds (HRAP) and PBRs and practical optimisation to be achieved (Chapter 2).

Objective 2. To determine whether microalgal character can be linked to nutrient removal abilities within low nutrient concentration environments (consistent with tertiary treatment) and inform species selection.

- A relationship between internal N:P (nitrogen:phosphorus) composition and nutrient remediation is evident and can be considered when selecting a species for nutrient remediation (Chapter 2 and 3).
- Species with a high N and low P internal composition (molar ratio > 18) remediated ammonium and P more efficiently (Chapter 3).
- Knowledge of this relationship and correlation to remediation performance, enables determination of the optimal species and required biomass concentration. When translated into an immobilised bead concentration, concentrations as low as 3.2 beads.mL^{-1} was found possible at a HRT of 20 h (Chapter 3).

Objective 3. To determine the critical operational parameters that influence the performance efficacy of IBR technology for tertiary nutrient removal.

- The external factors of light and temperature, in addition to species selection, are shown to have the greatest impact on growth and productivity of a microalgal bioreactor (Chapter 2).
- The relationship between microalgae and light is mostly unaffected by immobilisation, with immobilised microalgae withstanding greater light intensities (up to 1000 µmol.m^{-2}.s^{-1}) than suspended biomass (approximately 150 µmol.m^{-2}.s^{-1}), through light attenuation by the bead material (Chapter 5).
- Remediation performance and light conversion efficiency under a white light regime was greater than that under red or blue wavelengths (Chapter 5).

- Reducing the lighting periods through flashing and photoperiods, reduced the light energy demand but extended the overall treatment time, with constant light at 300 – 400 µmol.m^{-2}.s^{-1}determined optimal (Chapter 5).
- Effective treatment up to a maximum loading rate of 0.8 µgP.$bead^{-1}$$d^{-1}$ was found for immobilised *Scenedesmus obliquus* (Chapter 4).
- The effective cycle time of operation is linked to the total phosphate treated, with cycle times up to 24 days when operating at low loadings of 1.3 g.m^{-3}.d^{-1} consistent with polishing effluent from 1 mg.L^{-1} down to sub 0.3 mg.L^{-1} at a HRT of 20 h (Chapter 4).

Objective 4. To understand they key design and operating components that influence the overall economic viability of the technology and in doing so understand the potential for an IBR to be economically viable in comparison to traditional approaches.

- Design and economic analysis of an IBR for wastewater nutrient remediation identified the major cost items contributing to the whole life cost (WLC) included the operational costs of the LEDs and the unit cost of the LEDs and microalgal beads (Chapter 6).
- Predictions of a 50% reduction in LED unit cost reduces the CAPEX of an IBR to 66% of the estimated present day cost over the next 10 years (Chapter 6).
- No commercial supply chain for immobilised microalgae currently exists, with a future supply chain plausible in view of the market available for other immobilised microorganisms at a predicted cost of approximately £10.kg^{-1} of beads (Chapter 6).

- Historical trends in LED bulb development predict light output at a lower power consumption, with operational lighting costs approximately 30% of the estimated present day cost over the next 10 years (Chapter 6).
- Following predictions in cost reductions over the next decade, a WLC of approximately £0.8M is estimated for an IBR (including energy recovery through biomethane recovery of digested beads), comparable to WLC of conventional solutions (Chapter 6).

8.2 Future Work

In the course of this project further areas for research have been identified, these are listed below:

- Further development and optimisation of bead production is necessary to establish the potential of a commercial supply chain. Bead production within this thesis utilised the natural polymer, sodium alginate (Na-alginate). Na-alginate represents a major cost within the manufacture process at £33.04.kg^{-1} (Brenntag UK Ltd, 2015, pers. comm, 3rd July) opposed to £0.29.kg^{-1} $FeCl_3$. Substituting this material with a more economical option, either natural or artificial, has the potential to reduce production costs and facilitate manufacture and purchase through a commercial supply chain.
- The calcium-alginate (Ca-alginate) beads (Na-alginate plus a $CaCl_2$ curing solution) used during experimental analysis were found to degrade within some wastewater environments such that trials were halted through the release of microalgal biomass (Chapter 4). Methods of strengthening beads produced from natural polymers, through the addition of compounds to enhance the cross-linking formation or coating the bead with a microporous membrane layer (Kim et al., 2015), should be investigated with the impact of improved bead life re-assessed within an economic analysis.
- Bead regeneration and/or recovery should be investigated as a further method of improving affordability. Natural polymers such as Ca-alginate, can be dissolved after bead formation with sodium citrate used during the experimental analysis to determine the cell.bead^{-1} concentration. Such methodologies which enable recovery of the resin material for reuse should be investigated and re-assessed within an economic analysis.

- Indirect removal of P through precipitation was found to contribute between 33.3 – 88.5% of total recovery within lab scale batch trials. Methods to recover this precipitate, through modification of the reactor design similar to a Crystalactor®, should be considered to maximise resource recovery.
- The use of LED bulbs contributes significantly to the CAPEX and OPEX of an IBR. The current lighting system design is based on a light penetration depth determined for a packed bed scenario. The relative penetration depths under fluidisation needs further investigation to enable the lighting system to be appropriately designed.
- Biomethane potential (BMP) of the Ca-alginate beads during anaerobic digestion was determined during preliminary trials. Further trials should be completed to confirm these values (or the BMP value of an alternative resin selected through optimisation of bead manufacture), as the energy generation component of the process contributes significantly to the overall affordability (Chapter 6).
- Alternative uses for the immobilised microalgae post-treatment should be investigated which may further support economic viability, for instance application to land has demonstrated improved soil conditions (Trejo et al., 2012) with further investigation into the income generated through this route warranted as an alternative application for the beads post-treatment.
- The majority of the investigative work was completed using a single species, *Scenedesmus obliquus* chosen due to its popularity within the literature and ease of cultivation. Characterisation trials with alternative freshwater species found *Chlorella vulgaris* to be better suited to wastewaters with a varying influent concentration (Chapter 3), offering the potential for further improvements in performance which can be validated through further investigation.

- An advantage of the immobilisation process is the ability to control the community of species within the reactor. This enables scenarios which include; 1) immobilising differing species and mixing beads in differing quantities to create a tailored community and, 2) seeding an IBR reactor with microalgae characterised and suited to lower nutrient concentrations which follows an initial 'roughing' IBR. Such configurations may improve treatment time, efficiency and batch bead life and warrants further investigation.
- The majority of the experimental work was completed at a set temperature of 20^{o}C. It is acknowledged that this is unrepresentative of wastewater temperatures throughout the year, with telemetry data provided by the sponsoring companies reporting average effluent temperatures ranging from 6.4^{o}C to 13.5^{o}C for winter and summer months respectively. Preliminary remediation trials at 4^{o}C found similar results to those at 20^{o}C, however further investigation is required to confirm performance at lower temperatures.
- A larger pilot-scale IBR is required to validate long term performance throughout the year, with varying loads and seasonal temperatures, to enable translation of the results found within the laboratory and enable a better informed economic assessment.

8.2.1 References

Kim, S.R. et al. (2015), "Macroencapsulation of quorum quenching bacteria by polymeric membrane layer and its application to MBR for biofouling control", *Journal of Membrane Science*, vol. 473, pp. 109–117.

Trejo, A. et al. (2012), "Recycling waste debris of immobilized microalgae and plant growth-promoting bacteria from wastewater treatment as a resource to improve fertility of eroded desert soil", *Environmental and Experimental Botany*, vol. 75, pp. 65–73.

Appendices

Appendix A Microalgae for Municipal Wastewater Nutrient Remediation: Mechanisms, Reactors and Outlook for Tertiary Treatment – Supplementary Information

Table A.1 HRAP design parameters and performance data.

Design parameters		Algal community	Test conditions		Influent concentration ($mg.L^{-1}$)		Removal efficiency (%); Uptake rate		Effluent pH	References
HRT (d); Flow velocity ($m^3.d^{-1}$); Depth (m); Scale (m^3)	Paddle wheel velocity ($cm.s^{-1}$)		Test waters; Temp (ºC); pH	Irradiance ($\mu mol.m^{-2}.s^{-1}$) [a]	NH_4^+	TP	NH_4^+	TP		
3 0.16 0.3 0.47	9	*Dicyosphaerium pulchellum, Chlorella sp, Micratinium pusillum, Scenedesmus armatus. S.acutus*	Urban wastewater 27.3 --	685 – 2,879[a]	41.3	8.5	95.2 --	--	9.1	(García et al., 2000)
4 0.12 0.3 0.47	9		Urban wastewater 21.7 --	685 – 2,879[a]	41.3	8.5	84.5 --	--	9.0	(García et al., 2000)
4 0.12 0.3 0.47	9	*Dicyosphaerium pulchellum, Chlorella sp, Micratinium pusillum, Scenedesmus armatus. S.acutus*	Urban wastewater 27.3 --	685 – 2,879[a]	41.3	8.5	96.6 --	--	9.0	(García et al., 2000)
5 0.10 0.3 0.47	9		Urban wastewater 22.9 --	685 – 2,879[a]	41.3	8.5	93.7 --	--	9.2	(García et al., 2000)
7 0.067 0.3 0.47	9		Urban wastewater 21.7 --	685 – 2,879[a]	41.3	8.5	94.7 --	--	9.2	(García et al., 2000)

Design parameters		Algal community	Test conditions		Influent concentration ($mg.L^{-1}$)		Removal efficiency (%); Uptake rate		Effluent pH	References
HRT (d); Flow velocity ($m^3.d^{-1}$); Depth (m); Scale (m^3)	Paddle wheel velocity ($cm.s^{-1}$)		Test waters; Temp (ºC); pH	Irradiance ($\mu mol.m^{-2}.s^{-1}$) [a]	NH_4^+	TP	NH_4^+	TP		
7 0.067 0.3 0.47	9	*Dicyosphaerium pulchellum, Chlorella sp, Micratinium pusillum, Scenedesmus armatus. S.acutus*	Urban wastewater 22.9 --	685 – 2,879[a]	41.3	8.5	98.3 --	--	9.4	(García et al., 2000)
8 0.058 0.3 0.47	9	*Dicyosphaerium pulchellum, Chlorella sp, Micratinium pusillum, Scenedesmus armatus. S.acutus*	Urban wastewater 11.8 --	685 – 2,879[a]	41.3	8.5	92.3 --	--	8.6	(García et al., 2000)
10 0.047 0.3 0.47	9	*Dicyosphaerium pulchellum, Chlorella sp, Micratinium pusillum, Scenedesmus armatus. S.acutus*	Urban wastewater 11.8 --	685 – 2,879[a]	41.3	8.5	97.1 --	--	8.8	(García et al., 2000)
-- 360 0.2-0.5 200-500	5-30	*Micractinium dominant*	Primary effluent 10-25 7.1	225 – 1,360[a]	21	9	85.4 --	46.2[c] --	9-10	(Nurdogan and Oswald, 1995)
-- 360 0.2-0.5 200-500	5-30	*Micractinium dominant*	Primary effluent with CaO 10-25 7.1	225 – ,1360[a]	21	9	85 --	99[c]	10-11	(Nurdogan and Oswald, 1995)

Design parameters		Algal community	Test conditions		Influent concentration ($mg.L^{-1}$)		Removal efficiency (%); Uptake rate		Effluent pH	References
HRT (d); Flow velocity ($m^3.d^{-1}$); Depth (m); Scale (m^3)	Paddle wheel velocity ($cm.s^{-1}$)		Test waters; Temp (ºC); pH	Irradiance ($\mu mol.m^{-2}.s^{-1}$) [a]	NH_4^+	TP	NH_4^+	TP		
8 -- 0.35 16.8	--	--	Domestic wastewater 7.3-25.3 7.85	166 – 1,327[a]	27.3	14.7	74.6 --	41.5 --	8.5	(Picot et al., 1992)
9 486 0.35 4375	20	--	Primary effluent 7.2 9.3	--	20.0	1.8	53 --	22[d] --	--	(Sutherland et al., 2014)
7 486 0.35 4375	20	--	Primary effluent 13.0 9.7	--	22.1	2.1[d]	79 --	49[d] --	--	(Sutherland et al., 2014)
5.5 486 0.35 4375	20		Primary effluent 17.7 9.3	--	28.7	0.9[d]	77 --	20[d] --	--	(Sutherland et al., 2014)
7 486 0.35 4375	20		Primary effluent 12.5 9.0	--	30.7	3.6[d]	47 --	37[d] --	--	(Sutherland et al., 2014)
-- 500 $m^2.d^{-1}$ 0.35 4375	20		Primary effluent 13.2-14.3 7.6	--	24.2	1.92[d]	5.6-67.4	14-24.	9.1-9.3	(Craggs et al., 2012)

Design parameters		Algal community	Test conditions		Influent concentration ($mg.L^{-1}$)		Removal efficiency (%); Uptake rate		Effluent pH	References
HRT (d); Flow velocity ($m^3.d^{-1}$); Depth (m); Scale (m^3)	Paddle wheel velocity ($cm.s^{-1}$)		Test waters; Temp (ºC); pH	Irradiance ($\mu mol.m^{-2}.s^{-1}$) [a]	NH_4^+	TP	NH_4^+	TP		
4 0.14 0.25 0.55	20	*Phormidium sp.* *Anabeana sp.* *Chlorella sp.* *dominated*	Synthetic wastewater 20.5 --	1,215[a]	--	--	67.8[b] --	18 --	8.3	(Cromar and Fallowfield, 1997)
4 0.14 0.25 0.55	20		Synthetic wastewater 21.0 --	1,215[a]	--	--	74.7[b] --	69 --	8.8	(Cromar and Fallowfield, 1997)
7 0.08 0.25 0.55	20		Synthetic wastewater 18.9 --	818[a]	--	--	78.7[b] --	45.5 --	8.3	(Cromar and Fallowfield, 1997)
7 0.08 0.25 0.55	20		Synthetic wastewater 18.9	818[a]	--	--	65.6[b] --	92.9 --	9.1	(Cromar and Fallowfield, 1997)

[a] Irradiance units converted to $\mu mol.m^{-2}.s^{-1}$ using conversion guidelines within (Thimijan and Heins, 1983)
[b] Total Nitrogen
[c] Orthophosphate
[d] Dissolved reactive phosphorus (DRP)

Table A.2 Photobioreactor systems, design parameters and performance data.

Design parameters		Test conditions			Influent conc ($mg.L^{-1}$)		Removal efficiency (%)			
PBR description; Aeration ($L.min^{-1}$)	**HRT (d); Flow velocity ($m^3.d^{-1}$); Depth (cm); Scale (m^3)**	**Algae; Conc; Suspended / attached**	**Test waters; Temp (ºC); pH**	**Irradiance ($\mu mol.m^{-2}.s^{-1}$)[a]; Day length (h)**	**NH_4^+**	**TP**	**NH_4^+**	**TP**	**Specific growth rate; Biomass productivity ($g.SS.L^{-1}.d^{-1}$)**	**References**
Flat panel 2.8	3.3 Batch 4.4 0.0045	*S.obliquus* -- Suspended	Secondary effluent 20 7	250 14	31[a]	1.63	100[a]	100	0.94 0.23	(Ruiz et al., 2013)
Flat panel 2.8	3.4 0.01 4.4 0.0045	*S.obliquus* -- Suspended	Secondary effluent 20 7	250 14	17.7[a]	1.57	69.9[a]	94.9	-- 0.29	(Ruiz et al., 2013)
Flat panel 2.8	2.8 0.001 4.4 0.0045	*S.obliquus* -- Suspended	Secondary effluent 20 7	250 14	34.9[a]	3.56	86.8[a]	97.8	-- 0.38	(Ruiz et al., 2013)
Flat panel 2.8	2.3 0.001 4.4 0.0045	*S.obliquus* -- Suspended	Secondary effluent 20 7	250 14	15.2[a]	0.81	88.8[a]	90.1	-- 0.28	(Ruiz et al., 2013)
Flat panel 2.8	1.7 0.003 4.4 0.0045	*S.obliquus* -- Suspended	Secondary effluent 20 7	250 14	22.2[a]	2.14	91.0[a]	96.7	-- 0.36	(Ruiz et al., 2013)
Flat panel 2.8	1.1 0.004 4.4 0.0045	*S.obliquus* -- Suspended	Secondary effluent 20 7	250 14	19.7[b]	1.75	89.8[a]	94.9	-- 0.35	(Ruiz et al., 2013)

Design parameters		Test conditions			Influent conc ($mg.L^{-1}$)		Removal efficiency (%)			
PBR description; Aeration ($L.min^{-1}$)	**HRT (d); Flow velocity ($m^3.d^{-1}$); Depth (cm); Scale (m^3)**	**Algae; Conc; Suspended / attached**	**Test waters; Temp (°C); pH**	**Irradiance ($\mu mol.m^{-2}.s^{-1}$)[a]; Day length (h)**	**NH_4^+**	**TP**	**NH_4^+**	**TP**	**Specific growth rate; Biomass productivity ($g.SS.L^{-1}.d^{-1}$)**	**References**
Flat panel 2.8	0.5 0.009 4.4 0.0045	*S.obliquus* -- Suspended	Secondary effluent 20 7	250 14	16.6[a]	2.0	0[a]	0	-- 0	(Ruiz et al., 2013)
Tubular reactor (indoor) --	1 Batch 4.1 diameter 0.0151	*Scenedesmus* sp. -- Suspended	Secondary effluent 20 7.2-8.5	200 24	36.2	2.6	99.7	98.8	0.39 --	(Di Termini et al., 2011)
Tubular reactor (outdoor) --	7 Batch 4.1 diameter 0.0151	*Scenedesmus* sp. -- Suspended	Secondary effluent 20 --	Max 1,300 --	21.96	1.49	79.0	70.5	0.02 --	(Di Termini et al., 2011)
Semi-open --	-- Batch 10.2 1.5	*Chlorella* sp (wild type) -- Suspended	Centrate 26 6.2	25 --	275[c]	392	19.5[b]	58.1[c]	0.53 --	(Min et al., 2011)
Semi-open --	16 0.3 10.2 1.5	*Chlorella* sp (wild type) -- Suspended	Centrate 28.1 7.5	25 --	275[c]	392	11.9[b]	44.9[c]	--	(Min et al., 2011)
Semi-open --	23 0.4 10.2 0.0151	*Chlorella* sp (wild type) -- Suspended	Centrate 24.9 7.8	25 --	275[c]	392	41.2[b]	50.0[c]	--	(Min et al., 2011)
Semi-open --	9 0.6 10.2 1.5	*Chlorella* sp (wild type) -- Suspended	Centrate 27.9 7.0	25 --	275[c]	392	61.1[b]	60.9[c]	--	(Min et al., 2011)

Design parameters			Test conditions		Influent conc ($mg.L^{-1}$)		Removal efficiency (%)			
PBR description; Aeration ($L.min^{-1}$)	**HRT (d); Flow velocity ($m^3.d^{-1}$); Depth (cm); Scale (m^3)**	**Algae; Conc; Suspended / attached**	**Test waters; Temp (°C); pH**	**Irradiance ($\mu mol.m^{-2}.s^{-1}$)[a]; Day length (h)**	**NH_4^+**	**TP**	**NH_4^+**	**TP**	**Specific growth rate; Biomass productivity ($g.SS.L^{-1}.d^{-1}$)**	**References**
Semi-open CO_2 aeration	23 0.3 10.2 1.5	*Chlorella* sp (wild type) -- Suspended	Centrate 26.4 7.3	25 --	275[c]	392	19.7[b]	26.7[c]	--	(Min et al., 2011)
Semi-open CO_2 aeration	19 0.4 10.2 1.5	*Chlorella* sp (wild type) -- Suspended	Centrate 27.9 7.0	25 --	275[c]	392	45.5[b]	47.2[c]	--	(Min et al., 2011)
Semi-open CO_2 aeration	17 0.6 10.2 1.5	*Chlorella* sp (wild type) -- Suspended	Centrate 24.9 7.1	25 --	275[c]	392	45.5[b]	52.8[c]	--	(Min et al., 2011)
Tubular with support material for algal attachment 0.25	2.7 0.001 -- 0.035	*Chlorella vulgaris* 3 $g.L^{-1}$ Attached	Synthetic wastewater 26 6.5-7.0	36 20	10	--	79.0	-- --	--	(Karapinar Kapdan and Aslan, 2008)
Tubular with support material for algal attachment 0.25	2.7 0.001 -- 0.035	*Chlorella vulgaris* 3 $g.L^{-1}$ Attached	Synthetic wastewater 26 6.5-7.0	36 20	48	--	45.8	--	--	(Karapinar Kapdan and Aslan, 2008)

Design parameters		Test conditions			Influent conc ($mg.L^{-1}$)		Removal efficiency (%)			
PBR description; Aeration ($L.min^{-1}$)	**HRT (d); Flow velocity ($m^3.d^{-1}$); Depth (cm); Scale (m^3)**	**Algae; Conc; Suspended / attached**	**Test waters; Temp (°C); pH**	**Irradiance ($\mu mol.m^{-2}.s^{-1}$)[a]; Day length (h)**	**NH_4^+**	**TP**	**NH_4^+**	**TP**	**Specific growth rate; Biomass productivity ($g.SS.L^{-1}.d^{-1}$)**	**References**
Tubular with support material for algal attachment 250 $mL.min^{-1}$	1.7 0.02 -- 0.035	*Chlorella vulgaris* 3 $g.L^{-1}$ Attached	Synthetic wastewater 26 6.5-7.0	36 20	20	2.5	35	--	--	(Karapinar Kapdan and Aslan, 2008)
Tubular with support material for algal attachment 0.25	5.4 0.01 -- 0.035	*Chlorella vulgaris* 3 $g.L^{-1}$ Attached	Synthetic wastewater 26 6.5-7.0	36 20	20	2.5	93.0	--	--	(Karapinar Kapdan and Aslan, 2008)
Tubular with support material for algal attachment 0.25	5.5 0.001 -- 0.035	*Chlorella vulgaris* 3 $g.L^{-1}$ Attached	Synthetic wastewater 26 6.5-7.0	36 20	--	--	70.0	--	--	(Karapinar Kapdan and Aslan, 2008)
Tubular with support material for algal attachment 0.25	5.5 0.001 -- 0.035	*Chlorella vulgaris* 3 $g.L^{-1}$ Attached	Synthetic wastewater 26 6.5-7.0	36 20	10	--	93.0	--	--	(Karapinar Kapdan and Aslan, 2008)

[a] Total Nitrogen

[b] Total Kjeldahl Nitrogen (TKN)

[c] Orthophosphate

Table A.3 Microalgal biofilm systems, design parameters and performance data.

Design parameters		Algal community	Test conditions		Influent concentration ($mg.L^{-1}$)		Removal efficiency (%); Uptake rate ($mg.m^{-2}.d^{-1}$)		Biomass production ($g.m^{-2}.d^{-1}$) dry weight	References
Substrata; Area (m^2)	**HRT (d); Flow velocity ($m^3.d^{-1}$); Depth (m)**		**Test waters; Temp (ºC); pH**	**Irradiance ($\mu mol.m^{-2}.s^{-1}$)[a]**	**NH_4^+**	**TP**	**NH_4^+**	**TP**		
FLOWAY										
Floway periphyton scrubber: plastic sheets 11.5	-- 128 0.001-0.003	*Cladophora crispata, Enteromorpha micrococca, Stigeoclonium tenue, Cladphora* sp, *Spirogyra rivularis, Dichotomosiphon tuberosus, Eunotia pectinalis, Melsoria varians, Oscillatoria subbrevis, Cosmospogan coeruleus*	Agricultural run-off 18.1-27.2 7.7	--	--	0.058	--	17.0 124	21.2	(Adey et al., 1993)
Serial periphyton scrubber: plastic sheets 2.7	-- 37 --		Agricultural run-off 18.1-27.2 7.7	--	--	0.038	--	15.2 102	21.6	(Adey et al., 1993)
Algal Turf Scrubber Single Floway 1012	-- 436-1226 0.02-0.04	*Oscillatoria, Navicula* sp. *Nitzschia* sp., *Cyclotella* sp., *Ulothrix* sp., *Cladophora* sp., *Microspora* sp.	Secondary effluent 18.9 8.4	--	3.3	3.1	24.2 1,110[a]	45.2 730	35.0	(Craggs et al., 1996a; Craggs et al., 1996b; Craggs, 2001)
Flow lanes 0.0048	3 -- --	Community sampled from sedimentation tank	Modified BG11 20-30 --	15-120	--	--	--	-- 0.3-119.9	0.17-29.0	(Guzzon et al., 2008)

Design parameters		Algal community	Test conditions		Influent concentration ($mg.L^{-1}$)		Removal efficiency (%); Uptake rate ($mg.m^{-2}.d^{-1}$)		Biomass production ($g.m^{-2}.d^{-1}$) dry weight	References
Substrata; Area (m^2)	**HRT (d); Flow velocity ($m^3.d^{-1}$); Depth (m)**		**Test waters; Temp (ºC); pH**	**Irradiance ($\mu mol.m^{-2}.s^{-1}$)[a]**	**NH_4^+**	**TP**	**NH_4^+**	**TP**		
PVC sheet flow cell 1.8	0.006 0.0004-0.007 0.02	*Nitzchia* and green filamentous	Synthetic wastewater 22 7.0	230	--	--	-- 1[d]	-- 130	--	(Boelee et al., 2011)
Unglazed pre-soaked quarry tiles 12.2	-- -- 0.005-0.01	*Characium pringsheimii; Oedogonium; Palmellopsis gelatinosa; Pseudopleurococcus* sp*; Scenedesmus quadrucauda; Stigeoclonium; Ulothrix* plus other cyanobacteria and diatoms	Secondary effluent 11.9 --	270.3 $g.cal.cm^{-2}.d^{-1}$	--	--	-- 1,903[b]	-- 157	130 $g.m^{-2}$	(Davis et al., 1990a; Davis et al., 1990b)
Plastic mesh *(Periphyton-fish system)* 48	--	--	Secondary effluent -- --	--	--	--	82[c] 108[c]	23 27	--	(Rectenwald and Drenner, 2000)
SUBSTRATE SUBMERSION										
Rotating Algal Biofilm Reactor (RABR) 4.26	12 16.4 0.9	*Diatoma, Pediastrum, Chlorella* sp	Wastewater effluent 11.8 --	208	7.8	4.5	-- 14,100	-- 2,100	31.0	(Christenson and Sims, 2012)
Polyurethane foam 0.00045	-- 0.5 $L.L^{-1}.d^{-1}$ 0.26	*Chlorella* sp.	Pre-treated cattle manure --	53[a]	237.0	34.0[b]	66.9 --	64.1[e] --	--	(Travieso et al., 1996)

Design parameters		Algal community	Test conditions		Influent concentration ($mg.L^{-1}$)		Removal efficiency (%); Uptake rate ($mg.m^{-2}.d^{-1}$)		Biomass production ($g.m^{-2}.d^{-1}$) dry weight	References
Substrata; Area (m^2)	**HRT (d); Flow velocity ($m^3.d^{-1}$); Depth (m)**		**Test waters; Temp (ºC); pH**	**Irradiance ($\mu mol.m^{-2}.s^{-1}$)[a]**	**NH_4^+**	**TP**	**NH_4^+**	**TP**		
Polyurethane foam 0.00045	-- 1 $L.L^{-1}.d^{-1}$ 0.26	*Chlorella* sp.	Pre-treated cattle manure --	53[a]	237.0	34.0[b]	62.8 --	60.3[e] --	--	(Travieso et al., 1996)
Polyurethane foam 0.00045	-- 1.5 $L.L^{-1}.d^{-1}$ 0.26	*Chlorella* sp.	Pre-treated cattle manure --	53[a]	237.0	34.0[b]	59.5 --	57.6[e] --	--	(Travieso et al., 1996)
Polyurethane foam 0.00045	-- 2.0 $L.L^{-1}.d^{-1}$ 0.26	*Chlorella* sp.	Pre-treated cattle manure --	53[a]	237.0	34.0[b]	52.6 --	51.5[e] --	--	(Travieso et al., 1996)
Polyurethane foam 0.00045	-- 2.5 $L.L^{-1}.d^{-1}$ 0.26	*Chlorella* sp.	Pre-treated cattle manure --	53[a]	237.0	34.0[b]	47.2 --	48.0[e] --	--	(Travieso et al., 1996)
Polyurethane foam 0.00045	-- 2.5 $L.L^{-1}.d^{-1}$ 0.26	*Chlorella* sp.	Pre-treated cattle manure --	265-372[a]	237.0	34.0[b]	58.2 --	55.4[e] --	--	(Travieso et al., 1996)
Polyurethane foam 0.00045	-- 0.5 $L.L^{-1}.d^{-1}$ 0.26	*Scenedesmus* sp.	Pre-treated cattle manure --	53[a]	237.0	34.0[b]	60.6 --	59.2[e] --	--	(Travieso et al., 1996)
Polyurethane foam 0.00045	-- 1 $L.L^{-1}.d^{-1}$ 0.26	*Scenedesmus* sp.	Pre-treated cattle manure --	53[a]	237.0	34.0[b]	58.6 --	57.8[e] --	--	(Travieso et al., 1996)

Design parameters		Algal community	Test conditions		Influent concentration ($mg.L^{-1}$)		Removal efficiency (%); Uptake rate ($mg.m^{-2}.d^{-1}$)		Biomass production ($g.m^{-2}.d^{-1}$) dry weight	References
Substrata; Area (m^2)	**HRT (d); Flow velocity ($m^3.d^{-1}$); Depth (m)**		**Test waters; Temp (°C); pH**	**Irradiance ($\mu mol.m^{-2}.s^{-1}$)[a]**	**NH_4^+**	**TP**	**NH_4^+**	**TP**		
Polyurethane foam 0.00045	-- 1.5 $L.L^{-1}.d^{-1}$ 0.26	*Scenedesmus* sp.	Pre-treated cattle manure --	53[a]	237.0	34.0[b]	54.5 --	53.2[d] --	--	(Travieso et al., 1996)
Polyurethane foam 0.00045	-- 2.0 $L.L^{-1}.d^{-1}$ 0.26	*Scenedesmus* sp.	Pre-treated cattle manure --	53[a]	237.0	34.0[b]	48.5 --	48.3[e] --	--	(Travieso et al., 1996)
Polyurethane foam 0.00045	-- 2.5 $L.L^{-1}.d^{-1}$ 0.26	*Scenedesmus* sp.	Pre-treated cattle manure --	53[a]	237.0	34.0[b]	42.0 --	44.0[e] --	--	(Travieso et al., 1996)
Polyurethane foam 0.00045	-- 2.5 $L.L^{-1}.d^{-1}$ 0.26	*Scenedesmus* sp.	Pre-treated cattle manure --	265-372[a]	237.0	34.0[b]	53.4 --	50.8[e] --	--	(Travieso et al., 1996)
Polystyrene foam 0.0136	6 Batch --	*Chlorella* sp. Initial growth	Dairy manure wastewater 20 --	110-120	309	770	94.3 --	73.4 --	4.3	(Johnson and Wen, 2010)
Polystyrene foam 0.0136	10 Batch --	*Chlorella* sp. Initial growth	Dairy manure wastewater 20 --	110-120	309	770	97.0 --	90.0 --	2.6	(Johnson and Wen, 2010)

Design parameters		Algal community	Test conditions		Influent concentration ($mg.L^{-1}$)		Removal efficiency (%); Uptake rate ($mg.m^{-2}.d^{-1}$)		Biomass production ($g.m^{-2}.d^{-1}$) dry weight	References
Substrata; Area (m^2)	**HRT (d); Flow velocity ($m^3.d^{-1}$); Depth (m)**		**Test waters; Temp (°C); pH**	**Irradiance ($\mu mol.m^{-2}.s^{-1}$)[a]**	**NH_4^+**	**TP**	**NH_4^+**	**TP**		
Polystyrene foam 0.0136	15 Batch --	*Chlorella* sp. Initial growth	Dairy manure wastewater 20 --	110-120	309	770	98.7 --	93.0 --	1.7	(Johnson and Wen, 2010)
Polystyrene foam 0.0136	6 Batch --	*Chlorella* sp. Re-growth	Dairy manure wastewater 20 --	110-120	309	770	97.1 --	76.6 --	4.3	(Johnson and Wen, 2010)
Polystyrene foam 0.0136	10 Batch --	*Chlorella* sp. Re-growth	Dairy manure wastewater 20 --	110-120	309	770	99.9 --	70.8 --	2.6	(Johnson and Wen, 2010)
Polystyrene foam 0.0136	15 Batch --	*Chlorella* sp. Re-growth	Dairy manure wastewater 20 --	110-120	309	770	99.9 --	62.3 --	1.7	(Johnson and Wen, 2010)
Radial flexibility PVC fillers --	6 Batch 0.8	*Chlorella pyrenoidosa, Scenedesmus obliquus, Anabaena flosaque, Microcystis aeruginosa*	Artificial wastewater 24-29 8.0	47[a]	18.2	10.4	91.9 --	98.2 --	--	(Wei et al., 2008)
Radial flexibility PVC fillers --	24 0.005 0.8		Artificial wastewater 24-29 8.0	47[a]	12.3	9.0	82.4 --	95.4 --	--	(Wei et al., 2008)

[a] Irradiance units converted to µmol.m^{-2}.s^{-1} using conversion guidelines within (Thimijan and Heins, 1983)
[b] Total Kjeldahl Nitrogen (TKN)
[c] Total Nitrogen
[d] Nitrate
[e] Orthophosphate

Table A.4 Matrix-immobilised design parameters and performance data.

Design parameters			Test conditions		Influent concentration ($mg.L^{-1}$)		Removal efficiency (%); Uptake rate			
Resin: Reactor configuration	HRT (d); Scale (m^3); Flow velocity ($m^3.d^{-1}$)	Algae; Concentration	Test waters; Temp (°C); pH	Irradiance ($\mu mol.m^{-2}.s^{-1}$)[a]; Day length (h)	NH_4^+	TP	NH_4^+	TP	Specific growth rate (d^{-1}); Final pH	References
Na-alginate Packed bed	0.3 0.001 0.48	*Chlorella kessleri* 0.1 bead.mL^{-1}	Wastewater effluent 30 8	53[a] (Artificial) 13	31	6.85	59 --	61.8 --	-- 9.9	(Travieso et al., 1992; Travieso et al., 1996)
Na-alginate Packed bed	0.3 0.001 0.48	*Chlorella kessleri* 0.1 bead.mL^{-1}	Wastewater effluent 30 7.8	690[a] (Natural) 13	24.1	9.2	26.1 --	58.7 --	-- 9.5	(Travieso et al., 1992; Travieso et al., 1996)
Na-alginate	2.1 0.003 Batch	*Chlorella vulgaris* $3x10^5$ cells.$bead^{-1}$, 2.6 beads.mL^{-1}	Wastewater effluent 25 --	135 --	--	--	80.0 0.512 $\mu g.h^{-1}.10^{-6}$cells	53.3[c] 0.041 $\mu g.h^{-1}.10^{-6}$cells[b]	0.195 9.0-9.5	(Ruiz-Marin et al., 2010)
Na-alginate Packed bed	0.3 0.001 0.48	*Chlorella vulgaris* 0.1 bead.mL^{-1}	Wastewater effluent 30 7.8	690[a] (Natural) 13	24.1	9.2	76.3 --	69.6 --	-- 10.2	(Travieso et al., 1992; Travieso et al., 1996)
Na-alginate Packed bed	0.3 0.001 0.48	*Chlorella vulgaris* 0.1 bead.mL^{-1}	Wastewater effluent 30 8	53[a] (Artificial) 13	6.85	31	65.7 --	63.5 --	-- 10.0	(Travieso et al., 1992; Travieso et al., 1996)
Na-alginate Fluidised 10.8 $mL.min^{-1}$	0.3 0.001 0.48	*Chlorella vulgaris* 0.1 bead.mL^{-1}	Wastewater effluent 30 7.8	53[a] (Artificial) 13	24.1	9.2	81.7 --	70.7 --	-- 9.1	(Travieso et al., 1992; Travieso et al., 1996)

Design parameters		Test conditions			Influent concentration ($mg.L^{-1}$)		Removal efficiency (%); Uptake rate			
Resin: **Reactor configuration**	**HRT (d); Scale (m^3); Flow velocity ($m^3.d^{-1}$)**	**Algae; Concentration**	**Test waters; Temp (°C); pH**	**Irradiance ($\mu mol.m^{-2}.s^{-1}$)[a]; Day length (h)**	**NH_4^+**	**TP**	**NH_4^+**	**TP**	**Specific growth rate (d^{-1}); Final pH**	**References**
Na-alginate Fluidised 10.8 $mL.min^{-1}$	0.75 0.001 0.48	*Chlorella vulgaris* 0.1 $bead.mL^{-1}$	Wastewater effluent 30 8	53[a] (Artificial) 13	6.85	31	69.8 --	65.5 --	-- 9.5	(Travieso et al., 1992; Travieso et al., 1996)
Na-alginate Fluidised 10.8 $mL.min^{-1}$	0.75 0.001 0.48	*Chlorella kessleri* 0.1 $bead.mL^{-1}$	Wastewater effluent 30 7.8	690[a] (Natural) 13	24.1	9.2	34.0 --	62.0 --	-- 8.5	(Travieso et al., 1992; Travieso et al., 1996)
Na-alginate Fluidised 10.8 $mL.min^{-1}$	0.75 0.001 0.48	*Chlorella kessleri* 0.1 $bead.mL^{-1}$	Wastewater effluent 30 8	53[a] (Artificial) 13	31	6.85	63.9 --	64.4 --	-- 8.5	(Travieso et al., 1992; Travieso et al., 1996)
Na-alginate	2.1 0.003 Batch	*Scenedesmus obliquus* $3x10^5$ $cells.bead^{-1}$, 2.6 $beads.mL^{-1}$	Wastewater effluent 25 --	135 --	--	--	95.4 0.365 $\mu g.h^{-1}.10^{-6}$ cells	85.1[d] 0.033 $\mu g.h^{-1}.10^{-6}$ cells	0.110 --	(Ruiz-Marin et al., 2010)
Na-alginate	12 0.004 0.007	*C.vulgaris* 32% bead:effluent	Wastewater effluent 30 -	~33 (white) 24	7.1-10.4[b]	0.12-1.78[d]	~80.0 --	100 --	0.38 --	(Filippino et al., 2015)
Na-alginate	12 0.004 0.007	*C.vulgaris* 32% bead:effluent	Wastewater effluent 30 --	~ 33 (red) 24	7.1-10.4[b]	0.12-1.78[d]	20.0 --	60.0 --	0.81 --	(Filippino et al., 2015)

Design parameters			Test conditions		Influent concentration ($mg.L^{-1}$)		Removal efficiency (%); Uptake rate			
Resin: **Reactor configuration**	**HRT (d); Scale (m^3); Flow velocity ($m^3.d^{-1}$)**	**Algae; Concentration**	**Test waters; Temp (°C); pH**	**Irradiance ($\mu mol.m^{-2}.s^{-1}$)[a]; Day length (h)**	**NH_4^+**	**TP**	**NH_4^+**	**TP**	**Specific growth rate (d^{-1}); Final pH**	**References**
Na-alginate	12 0.004 0.007	*C.vulgaris* 32% bead:effluent	Wastewater effluent 30 --	~33 (red) 24	7.1-10.4[b]	0.12-1.78[d]	100 --	70.0 --	1.44 7-7.5	(Filippino et al., 2015)
Na-alginate	6.5 0.004 0.012	*C.vulgaris* 32% bead:effluent	Wastewater effluent 30 7-7.5	~33 (red) 24	7.1-10.4[b]	1.9[c]	100.0 --	<10.0 --	1.10 7-7.5	(Filippino et al., 2015)
Na-alginate	6.5 0.004 0.012	*C.vulgaris* 32% bead:effluent	Wastewater effluent 30 7-7.5	~33 (red) 24	7.1-10.4[b]	0.12-1.78[d]	90-100 --	80.0 --	0.51 7-7.5	(Filippino et al., 2015)
Na-alginate	6.5 0.004 0.012	*C.vulgaris* 32% bead:effluent	Wastewater effluent 20 7-7.5	~33 (red) 24	7.1-10.4[b]	0.12-1.78[d]	-100 --	100 --	1.55 7-7.5	(Filippino et al., 2015)
Na-alginate	6.5 0.004 0.012	*C.vulgaris* 10% bead:effluent	Wastewater effluent 20 7-7.5	~33 (red) 24	7.1-10.4[b]	0.12-1.78[d]	80.0[c] --	--	1.14 7-7.5	(Filippino et al., 2015)
Na-alginate	2.1 0.003 Batch	*Chlorella vulgaris* $3x10^5$ cells.bead^{-1}, 2.6 beads.mL^{-1}	Artificial wastewater 25 --	135 --	32.5	2.5[d]	80.0 0.512 $\mu g.h^{-1}.10^{-6}$.cells[b]	53.3[c] 0.041 $\mu g.h^{-1}.10^{-6}$.cells[b]	0.183 9.0-9.5	(Ruiz-Marin et al., 2010)

Design parameters		Test conditions			Influent concentration ($mg.L^{-1}$)		Removal efficiency (%); Uptake rate			
Resin: Reactor configuration	HRT (d); Scale (m^3); Flow velocity ($m^3.d^{-1}$)	Algae; Concentration	Test waters; Temp (ºC); pH	Irradiance ($\mu mol.m^{-2}.s^{-1}$)[a]; Day length (h)	NH_4^+	TP	NH_4^+	TP	Specific growth rate (d^{-1}); Final pH	References
Na-alginate	2 0.005 Batch	*Chlorella vulgaris* $1x10^6$ cells.$bead^{-1}$, 3.89 beads.mL^{-1}	Artificial wastewater -- 7.5	174 --	--	--	76.2 --	86.7 --	--	(Tam and Wong, 2000)
Na-alginate	2 0.005 Batch	*Chlorella vulgaris* $1x10^6$ cells.$bead^{-1}$, 7.79 beads.mL^{-1}	Artificial wastewater -- 7.5	174 --	--	--	95.1 --	93.5 --	--	(Tam and Wong, 2000)
Na-alginate	2 0.005 Batch	*Chlorella vulgaris* $1x10^6$ cells.$bead^{-1}$, 11.68 beads.mL^{-1}	Artificial wastewater -- 7.5	174 --	--	--	100 --	93.9 --	--	(Tam and Wong, 2000)
Na-alginate	2 0.005 Batch	*Chlorella vulgaris* $1x10^6$ cells.$bead^{-1}$, 15.58 beads.mL^{-1}	Artificial wastewater -- 7.5	174 --	--	--	79.6 --	91.2 --	--	(Tam and Wong, 2000)
Na-alginate	2 0.005 Batch	*Chlorella vulgaris* $1x10^6$ cells.$bead^{-1}$, 19.47 beads.mL^{-1}	Artificial wastewater -- 7.5	174 --	--	--	83.5 --	91.5 --	--	(Tam and Wong, 2000)
Alginate	-- -- Batch	*Chlorella vulgaris* --	Chu-10 medium 26 6.8	72 14	--	--	-- 9.9 $\mu g.h^{-1}$	--	--	(Mallick and Rai, 1994)

Design parameters		Test conditions			Influent concentration ($mg.L^{-1}$)		Removal efficiency (%); Uptake rate			
Resin: **Reactor configuration**	**HRT (d); Scale (m^3); Flow velocity ($m^3.d^{-1}$)**	**Algae; Concentration**	**Test waters; Temp (°C); pH**	**Irradiance ($\mu mol.m^{-2}.s^{-1}$)[a]; Day length (h)**	**NH_4^+**	**TP**	**NH_4^+**	**TP**	**Specific growth rate (d^{-1}); Final pH**	**References**
Agar	-- -- Batch	*Chlorella vulgaris* --	Chu-10 medium 26 6.8	72 14	--	--	-- 9.6 $\mu g.h^{-1}$	-- 19.8 $\mu g.h^{-1}$[d]	--	(Mallick and Rai, 1994)
Carrageenan	-- -- Batch	*Chlorella vulgaris* --	Chu-10 medium 26 6.8	72 14	--	--	-- 10.6 $\mu g.h^{-1}$	-- 18.9 $\mu g.h^{-1}$[d]	--	(Mallick and Rai, 1994)
Chitosan	-- -- Batch	*Chlorella vulgaris* --	Chu-10 medium 26 6.8	72 14	--	--	-- 10.1 $\mu g.h^{-1}$	-- 22.4 $\mu g.h^{-1}$[d]	--	(Mallick and Rai, 1994)
Na-alginate	2.1 0.003 Batch	*Scenedesmus obliquus* $3x10^5$ cells.bead^{-1}, 2.6 beads.mL^{-1}	Artificial wastewater 25 --	135 --	32.5	2.5[d]	96.6 0.621 $\mu g.h^{-1}.10^{-6}$cells[b]	55.2[d] 0.041 $\mu g.h^{-1}.10^{-6}$ cells	0.157 --	(Ruiz-Marin et al., 2010)
Ca-alginate	9 0.0008 Batch	*Scenedesmus intermedius* 15.73 µg Chl a, 3.2 beads.mL^{-1}	BBM Medium 20 8-9	120 14	--	--	-- 9 $\mu g.h^{-1}$[b]	-- 1.2 $\mu g.h^{-1}$	0.011 mg Chl.h^{-1} --	(Jiménez-Pérez et al., 2004)
Ca-alginate	9 0.0008 Batch	*Nannochloris* sp 15.95 µg Chl a	BBM medium 20 8-9	120 14	--	--	-- 6 $\mu g.h^{-1}$[b]	-- 9 $\mu g.h^{-1}$	0.018 mg.Chl.h^{-1} --	(Jiménez-Pérez et al., 2004)

Design parameters			Test conditions		Influent concentration ($mg.L^{-1}$)		Removal efficiency (%); Uptake rate			
Resin: **Reactor configuration**	**HRT (d); Scale (m^3); Flow velocity ($m^3.d^{-1}$)**	**Algae; Concentration**	**Test waters; Temp (°C); pH**	**Irradiance ($\mu mol.m^{-2}.s^{-1}$)[a]; Day length (h)**	**NH_4^+**	**TP**	**NH_4^+**	**TP**	**Specific growth rate (d^{-1}); Final pH**	**References**
Alginate	1 -- Batch	*Anabaena doliolum* --	Medium of Allen & Arnon 26 7.5	72 14	--	--	-- 8.9 $\mu g.h^{-1}$	-- --	--	(Mallick and Rai, 1994)
Agar	1 -- Batch	*Anabaena doliolum* --	Medium of Allen & Arnon 26 7.5	72 14	--	--	-- 9.4 $\mu g.\ h^{-1}$	-- 17.5 $\mu g.h^{-1d}$	--	(Mallick and Rai, 1994)
Carrageenan	1 -- Batch	*Anabaena doliolum* --	Medium of Allen & Arnon 26 7.5	72 14	--	--	-- 10.2 $\mu g.\ h^{-1}$	-- 15.2 $\mu g.\ h^{-1d}$	--	(Mallick and Rai, 1994)
Chitosan	1 -- Batch	*Anabaena doliolum* --	Medium of Allen & Arnon 26 7.5	72 14	--	--	-- 9.6 $\mu g.\ h^{-1}$	-- 21.3 $\mu g.h^{-1d}$	--	(Mallick and Rai, 1994)

[a] Irradiance units converted to $\mu mol.m^{-2}.s^{-1}$ using conversion guidelines within (Thimijan and Heins, 1983)

[b] Total Nitrogen

[d] Nitrate

[d] Orthophosphate

Appendix B Microalgal Medium Recipes for Cultivation

B.1 Jaworski's Medium (JM)

1 mL of each stock solution made up to 1 L with DI water.

1. 4.0g.200 mL^{-1} $Ca(NO_3)_2$ $4H_2O$
2. 2.49g.200 mL^{-1} KH_2PO_4
3. 10.0g.200 mL^{-1} $MgSO_4$ $7H_2O$
4. 3.18g.200 mL^{-1} $NaHCO_3$
5. 0.45g EDTAFeNa and 0.45g $EDTANa_2$ in.200 mL^{-1}
6. 0.496g H_3BO_3, 0.278g $MnCl_2$ $4H_2O$, and 0.20g $(NH_4)6Mo_7O_{24}$ $4H_2O$ in 200 mL^{-1}
7. 0.008g Cyanocobalamin, 0.008g Thiamine HCl and 0.008g Biotin in 200 mL^{-1}
8. 16.0g.200 mL^{-1} $NaNO_3$
9. 7.2g.200 mL^{-1} Na_2HPO_4 $12H_2O$

B.2 Blue-Green Medium (BG11)

100 mL stock 1, 10 mL of stocks 2 – 8 and 1 mL of stock 9 made up to 1 L with DI water and adjusted to pH 7.1 with 1 M NaOH or HCl.

1. 15.0g.1000 mL^{-1} $NaNO_3$
2. 2.0g.500 mL^{-1} K_2HPO_4
3. 3.75 g.500 mL^{-1} $MgSO_4$ $7H_2O$
4. 1.80 g.500 mL^{-1} $CaCl_2$ H_2O
5. 0.30 g.500 mL^{-1} Citric acid
6. 0.30 g.500 mL^{-1} Ammonium ferric citrate green
7. 0.05g.500 mL^{-1} $EDTANa_2$
8. 1.00g.500 mL^{-1} Na_2CO_3
9. 2.86g H_3BO_3, 1.81 g $MnCl_2$ $4H_2O$; 0.39 g $ZnSO_4$ $7H_2O$, 0.08g $CuSO_4$ $5H_2O$ and 0.05g $Co(NO_3)_2$ $6H_2O$ in 1000 mL^{-1}

Appendix C Influence of Microalgal N and P Composition on Wastewater Nutrient Remediation – Supplementary Information

C.1 Bead production and calcium-alginate adsorption capacity methodology

Blank beads, containing no microalgae, were prepared to evaluate the adsorption capacity of Ca-alginate resin and its contribution to the overall remediation of NH_4^+ and PO_4^{3-}. Beads were prepared following the method of Ruiz-Marin et al., (2010) for a final 2% Na-alginate concentration and solidified within 2% $CaCl_2$. The resin solution was passed through a peristaltic pump and dropped into a magnetically stirred $CaCl_2$ solution from a height of 30 cm producing approximately 4,000 beads per 100 mL resin with a bead volume of 0.025 mL. Beads with an approximate diameter of 3 mm were formed and left within the $CaCl_2$ solution overnight. Prior to use the beads were rinsed several times with DI water.

The adsorption capacity of the Ca-alginate beads for NH_4-N and PO_4-P was examined in batch trials following the method of Gotah et al., (2004), where 5 g of beads were immersed in 150 mL of water supplemented with NH_4-N or PO_4-P at concentrations varying from 0.5 to 10 $mg.L^{-1}$. The initial pH was corrected to 7 and maintained using 0.1 M NaOH and HCl. Reactors were placed on a shaker table at 80 rpm and samples withdrawn over 160 mins. Residual concentrations were analysed in duplicate using Spectroquant test kits (Merck Millipore) and read via a Spectroquant Nova 60 spectrophotometer.

The quantity of the target nutrient adsorbed on the bead was determined using (Equation 8-1). Where q is the resin adsorption capacity ($mg.g^{-1}$), V the solution volume (L), M the weight wet of the beads (g) and C_i and C_f the initial and final residual concentrations ($mg.L^{-1}$) respectively. Results were plotted and the resin adsorption capacity estimated using a Freundlich isotherm.

$$q = \frac{V}{M}\left(C_i - C_f\right) \qquad \text{Equation 8-1}$$

C.2 Calcium-alginate resin adsorption capacity

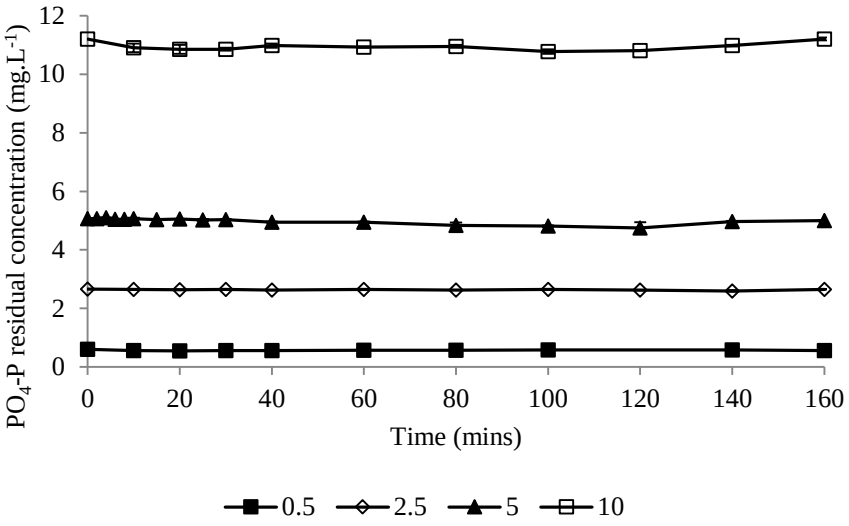


Figure A.1 PO_4-P remediation by blank calcium-alginate beads at initial concentrations of 0.5 (■), 2.5 (◊), 5 (▲) and 10 mg.L^{-1} (□).

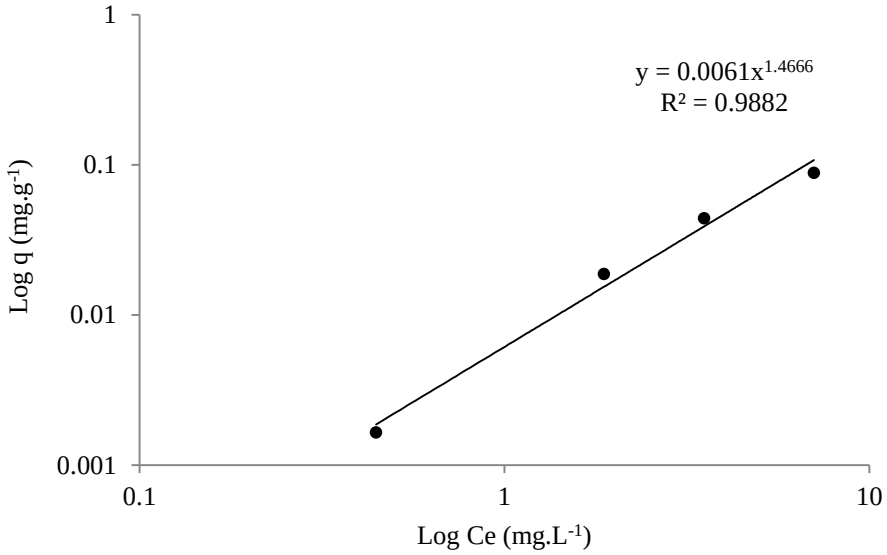


Figure A.2 Freundlich isotherm for calcium-alginate adsorption of NH_4-N.

Appendix D The Effect of Light Regime on Wastewater Nutrient Remediation by Immobilised Microalgae – Supplementary Information

D.1 Experimental set up and light regime

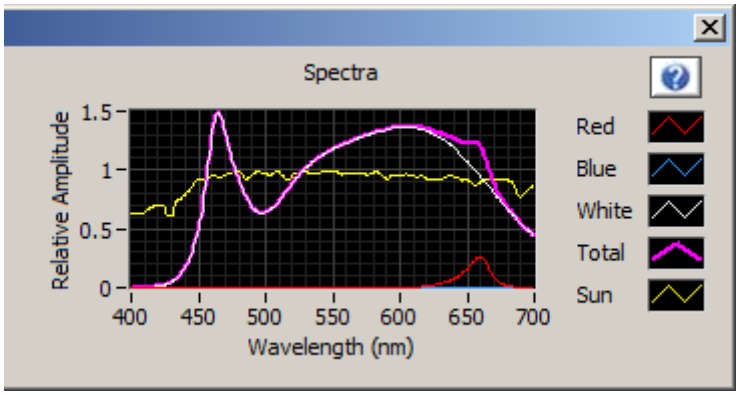


Figure A.3 Emission spectra of the LED panel with the Algem™ Labscale Photobioreactor.

D.2 Impact of wavelength and PFD on nutrient remediation and growth

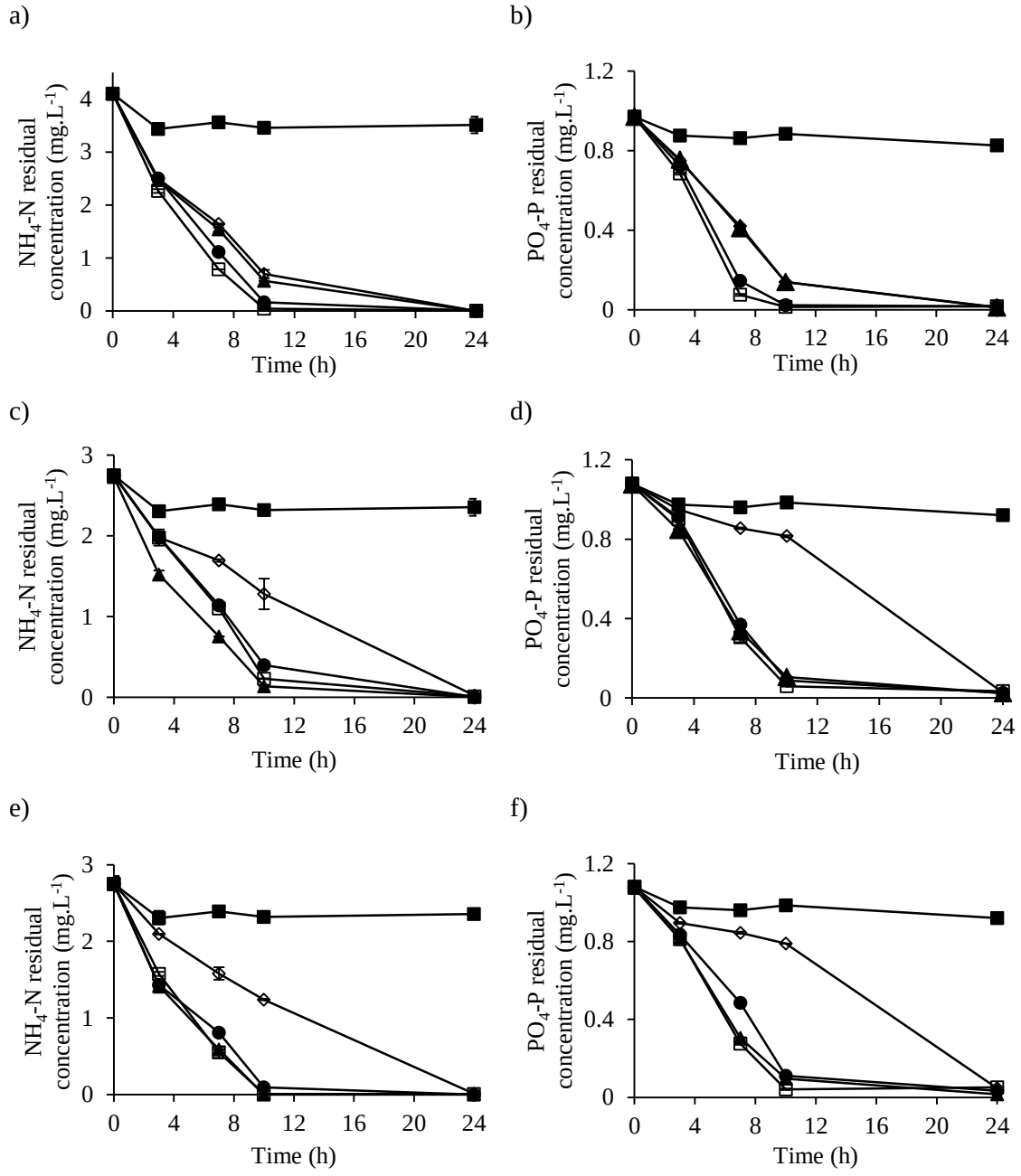


Figure A.4 Residual nutrient concentration for a) NH_4-N and b) PO_4-P under white light (400 – 700 nm); c) NH_4-N and d) PO_4-P under red light (660 nm); and e) NH_4-N and f) PO_4-P under blue light (465 nm). (■) 0, (◊) 50, (▲) 200, (●) 500 and (□) 1000 µmol.m^{-2}.s^{-1}.

Appendix E Implementation Challenges and Economic Assessment – Supplementary Information

E.1 IBR design

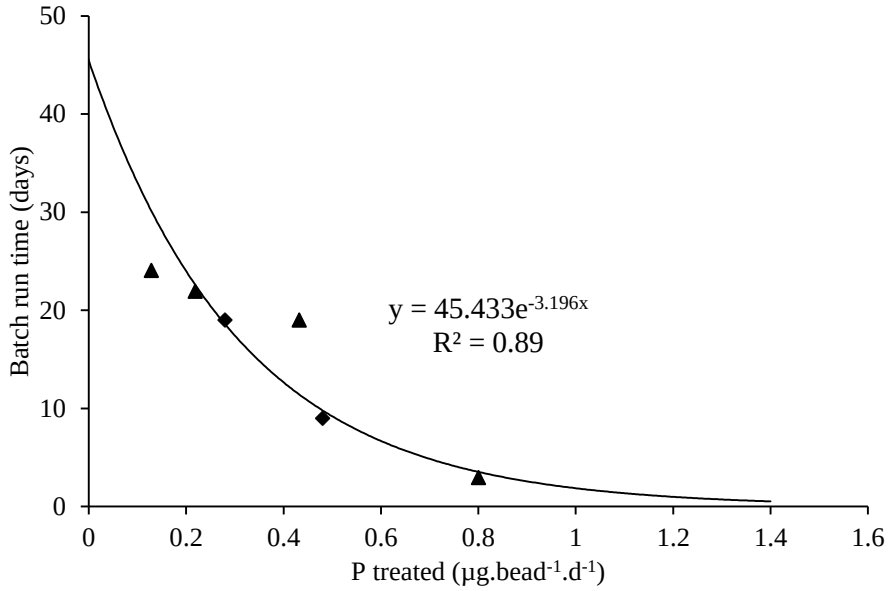


Figure A.5 Predicted bead batch length (days) with bead uptake rate as described in Chapter 4.

E.2 Design calculations

E.2.1 Scenario A and B: Coagulation

OVERALL SUMMARY

	Value	Unit
OPEX		
Chemical	4109.45	£.yr-1
Energy use	0.08	kWh.yr-1
Elec cost	0.01	£.yr-1
Coag transport cost	60.83	£.yr-1

DESIGN CALCULATIONS

	Unit	Value
Flow	360.00	m3.d-1
PE	2000.00	

Fe dosing

	Value	Unit	Notes	Reference
P initial	1.00	mg.L-1		
P consent	0.10	mg.L-1		
Difference	0.90	mg.L-1		
P removed	90.00	%		
Fraction P remaining	0.10			
Molar ratio Fe:P	7.42			Hauduc et al., (2015)
P	31.00	g.mol-1		
FeCl3	162.20	g.mol-1		
Fe	55.80	g.mol-1		
Coagulant strength	0.36	%	Assumption	

Specific gravity	1.36	kg.L-1	Ferric Chloride density	
FeCl3 cost	290.00	£.ton-1		
Fe3+ required	13.36	mg.L-1 - Fe		
FeCl3 dose required	107.84	mg.L-1		
	38.82	kg.d-1		
	28.46	L.d-1		
	10388.95	L.yr-1		
Cost	11.26	£.d-1		
	4109.45	**£.yr-1**		
Tankers per year	1.04	Unit		
Cost per year	**60.83**	**£**		

Dosing pump

	Unit	**Value**	**Notes**	**Reference**
Head	1.00	m	Assumption	
Pump efficiency (n)	0.50	%	Assumption	
			E = (Q.p.g.h)/(1000.n) kW	
FeCl3 Q	0.03	m3.d-1		
	0.00	m3.s-1		
p	1364.00	kg.m-3	Fluid density of FeCl3 - Ferric chloride density pdf, 20oC, 0.36 %	
g	9.81	m.s-1		
E	0.000009	kW		
Operation	8760.00	h.yr-1		
	0.08	kWh.yr-1		
Cost	**0.01**	£		

Sludge production Assuming all the P removed reacts to form ferric phosphate, FePO4

	Unit	Value	Notes	Reference
FePO4	150.60	g.mol-1		
FePO4	4.37	mg.L-1		
	1.57	kg.d-1		
			Remaining Fe(III) reacts with the alkalinity producing Ferric Hydroxide	
Fe(OH)3	106.80	g.mol-1		
Fe(OH)3	22.46	mg.L-1		
	8.09	kg.d-1		
Total sludge production	26.83	mg.L-1		
	9.66	kg.d-1		
	3526.08	kg.yr-1		
Sludge density	1650.00	kg.m-3	Cost+Assumptions	
	0.47	m3.yr-1		
	467.94	L.yr-1		

E.2.2 Scenario A: Coagulation and sand filtration

OVERALL SUMMARY

	Value	Unit
OPEX		
Energy use	3,986.67	kWh.yr-1
Elec cost	338.87	£.yr-1
CAPEX	1,119,803.97	£
Fixed Capital	**1,119,803.97**	**£**

ASSUMPTIONS/OMISSIONS

Sludge transport off site - assume capacity available for transportation with primary sludge with no extra transportation costs

DESIGN CALCULATIONS

	Value	Unit
Flow	360.00	m3.d-1
PE	2,000.00	

	Value	Unit	Notes	Reference
No. of filters	1.00	un		
Flow per filter	360.00	m3.d-1		
Area per filter	3.00	m2		
Overflow rate	5.00	m.h-1	Based on BluePro	
CAPEX	**1,008,913.46**	**£**		
Enough land?	TRUE	£		

Civils

	Value	Unit	Notes	Reference
Excavation depth	0.60	m		
Spacing around reactor	1.50	m		
Area	2.25	m2		
Excavation volume	1.35	m3		
Excavation cost	4.71	£		
Land preparation	0.48	£		
Concrete plynth	125.62	£		
Total	**130.81**	**£**		

Hydraulics

	Value	Unit	Notes	Reference
Filter feed pump	0.02	kWh.m-3	Specific energy consumption	
Filtration	0.01	kWh.m-3	Specific energy consumption	
Filter feed pump	2,628.00	kWh.yr-1		
Filtration	1,314.00	kWh.yr-1		
Total	**3,942.00**	kWh.yr-1		
Filter feed pump power	0.30	kW		
Filtration feed pump power	0.15	kW		

Coagulant dosing

	Value	Unit	Notes	Reference
FeCl3 dose required	28.46	L.d-1	Calculated	A_Coag
	10,388.95	L.yr-1		
Days storage	30.00	d		
Storage capacity	853.89	L		
	0.85	m3		
Dosing pump	0.00	kW	Calculated	A_Coag
Pump cost	**110,759.69**	**£**	Storage tank, transfer pumps, metering pumps, piping, valves and facility enclosure. Based on liquid Al feed. Remove below? (x2 as two point dosing system)	McGivney, W.T. (2008), pg 39

Sludge production and removal

Total sludge production	3,526.08	kg.yr-1	Calculated	A_Coag
Sludge density	1,650.00	kg.m-3		A_Coag
	0.47	m3.yr-1		A_Coag
	467.94	L.yr-1		A_Coag
	1.28	L.d-1	Assume capacity avaiable for transportation off site with primary sludge.	

E.2.3 Scenario B: Coagulation and aerated wetland

OVERALL SUMMARY

	Value	Unit
OPEX		
Energy use	65,100.70	kWh.yr-1
Elec cost	5,533.56	£.yr-1
CAPEX	846,237.45	£
Fixed Capital	846,237.45	£

DESIGN CALCULATIONS

	Value	Unit
Flow	360.00	m3.d-1
PE	2,000.00	

	Value	Unit	Notes	Reference
AW energy demand	0.49	kWh.m-3	Process energy demand	Ausitn and Nivala, (2009)
	64,386.00	kWh.yr-1		
Footprint	0.50	m2.PE-1		
	1,000.00	m2		
Enough land available	FALSE			
Additional land needed	800.00	m2		
Total land cost	**33,600.00**	**£**		
Aerated Wetland CAPEX	350.94	£.PE-1	Assume cost includes land preparation and installation of aeration system.	
CAPEX	**701,877.76**	£		

Coagulant dosing

	Value	Unit	Notes	Reference
FeCl3 dose required	28.46	L.d-1	Calculated	A_Coag
	10,388.95	L.yr-1		
Days storage	30.00	d		
Storage capacity	853.89	L		
	0.85	m3		
Dosing pump	0.01	kW	Calculated	A_Coag
Pump cost	**110,759.69**	**£**	Storage tank, transfer pumps, metering pumps, piping, valves and facility enclosure. Based on liquid Al feed. (x2 as two point dosing system)	McGivney, W.T. (2008), pg 39

Hydraulic pumping

Influent E = Q.p.g.h/(1000.n)

	Value	Unit	Notes	Reference
Q	0.004	m3.s-1		
p	998.00	kg.m-3		
g	9.81	m.s-2		
h	1.00	m	From underground drain of settlement tank to level of AW.	
n	0.50	%		
E	0.08	kW		
	714.70	kWh.yr-1		

E.2.4 Scenario C: High rate algal pond (HRAP)

ASSUMPTIONS/OMISSIONS

Anaerobic digestor available with capacity for microalgal feedstock with a suitable upfront pre-treatment
Coagulant dose based on S.obliquus (same species used for IBR)
Biomass transport off site, assume capacity available for additional produced biomass for transportation off sire with primary sludge with no extra transportation costs

DESIGN CALCULATIONS

	Value	Unit	Notes	Reference
Total Q	360.00	m3.d-1	2000 PE	
No. of ponds	8.00	un		
L	88.25	m	10 - 300 m	Ben-Amotz, 2008
Channel width	7.50	m	1 - 20 m	Ben-Amotz, 2008
Channel depth	0.30	m	Light paper - 1000 umol.m-2.s-1 (assuming approx. daylight intensities) to maintain a limit of 50 umol.m-2.s-1.	Thesis, chapter 5
	397.13	m3 per pond		
Distal	53.01	m3		
	3,601.12	total m3		
Surface area	12,003.72	m2		
Enough land	FALSE			
Additional land needed	11,803.72	m2		
Land cost	**495,756.10**	**£**		

Headloss		hb = (K.v^2)/2.g	Bends	Rogers et al., (2014)
	Value	**Unit**	**Notes**	**Reference**
K	2.00		Kinetic loss efficient 180^{o} bends	Rogers et al., (2014)
v	0.30	m.s-1		Amotz presentation
g	9.81	m.s-2		
hb	0.01		One bend - calc	
	0.02		Two bends - calc	

Friction loss across the length of the raceway

Mannings equation hc = v^2.n^2.(L/R^(4/3)) Channel Rogers et al., (2014)

	Value	Unit	Notes	Reference
n	0.01		Smooth plastic on granular earth	Algal culturing book
L	88.25	m	Design/calc	
R	0.28	m	Cross section of flow/wetted perimeter	
hc	0.01		One channel - calc	
	0.01		Two channels - calc	
Total headloss	0.03		Calc	

Paddlewheel W = 9.8.(Q.w.h/e) Rogers et al., (2014)

	Value	Unit	Notes	Reference
Q	0.68	m3.s-1	Considering 30 cm.sec-1 mixing speed	
w	998.00	kg.m3	Unit mass of water	
h	0.03		Total headloss	
e	0.17	%	Efficiency of paddle wheel - assumption	Algal culturing book
W	1,202.66	W	One pond - calc	
	9,621.31	W	All ponds - calc	
	10,535.34	kWh.yr-1	One pond - 24/7 operation	
	84,282.71	kWh.yr-1	All ponds - 24/7 operation	
	2.67	W.m-3		

Hydraulic pumping

Influent E = Q.p.g.h/(1000.n)

	Value	Unit	Notes	Reference
Q	0.00	m3.s-1		
p	998.00	kg.m-3		
g	9.81	m.s-2		
h	2.10	m	From underground drain of settlement tank to height of pond - assumption.	
n	0.50	%		
E	0.17	kW		
	1,500.87	kWh.yr-1		

Effluent E = Q.p.g.h/(1000.n) One pump for each pairing of ponds - 4 sets of 2.

	Value	Unit	Notes	Reference
Volume	45.00	m3.d-1	per pond	
	90.00	m3.d-1	two ponds coupled together configuration - need 4 pumps	
Pumps req	4.00	un		
Q	0.00	m3.s-1		
p	998.00	kg.m-3		
g	9.81	m.s-2		
h	2.00	m	To height of harvesting unit, DAF, assumption?	
n	0.50	%		
E	0.04	kW	per pump	
	1,429.40	kWh.yr-1	all pumps	

CAPEX

	Value	Unit	Notes	Reference
Paddle wheel				
Paddle motor	1.74	kW		
	2.00	kW	motor	
No. of motors	8.00	un	One motor/paddle wheel for two ponds - one paddlewheel for 1,500 m2!	
Paddlewheel	12,076.42	£	Cost sheet	Rogers et al., (2014)
Cost	**96,611.36**	£	Total	
Excavation and land preparation				
Per pond	397.13	m3		
Total	3,177.00	m3		
Cost.m-3	3.49	£	Cost sheet	
	11,084.44	£	Total	
Land preparation				
Cost.m-2	0.21	£	Cost sheet	
	2,569.36	£	Total	
Outside walls and internal island				
Outside walls				
Wall depth	0.30	m	Around edge of excavation	
Wall thickness	0.30	m	Assumption	
	20.13	m3	per pond	

Island				
Wall depth	0.60	m	From base to level with outside walls	
Length	88.25	m		
	15.89	m3	per pond	
Total concrete	36.01	m3	per pond	
	288.09	m3	all ponds	
Cost.m-3	93.05	£.m-3	Cost sheet	
	26,807.44	£	Total	
Pond Liner			Life span? For considering replacement?	
Height above ground	0.60	m		
Channel liner	767.78	m2		
Distal	206.12	m2		
Total liner area	973.89	m2	per pond	
	7,791.16	m2	all ponds	
	8,570.28	m2	plus 10%	
	8,500.00	m2	Round up	
Cost.m-2	4.57	£	Cost sheet	
	38,807.00	£	Total	
Hydraulics				
Pumps				
Influent pump	2,767.38	£	Cost sheet	
No. req	1.00	un		
	2,767.38	£	Total	
Effluent pump	2,767.38	£	Cost sheet	
No. req	4.00	un		
	11,069.53	£	Total	

BIOMASS RECOVERY AND INCOME

Coagulant

	Value	Unit	Notes	Reference
FeCl3 dose	150.00	mg.L-1	Assuming 36% strength and same specifci gravity as A_Coag, value based on mono-culture of S.obliquus grown within a HRAP	Chen et al., (2013)
	54.00	kg.d-1		
Specific gravity	1.36	kg.L-1		
	39.71	L.d-1	1.654411765	
	14,492.65	L.yr-1		
	14.49	m3.yr-1		
Storage tank	2.42	m3	Topped up every 2 months	
Storage tank	**2,744.73**	**£**		
Harvesting efficiency	97.30	%		Chen et al., (2013)
Harvesting daily rate	10.00	%		Rogers et al., (2014)
Daily removed	36,000.00	L.d-1	Calculated	
	5.40	kg.d-1	Calculated	
Cost	**571.59**	**£.yr-1**		
Biomass concentration	0.20	g(DW).L-1	Estimated value reported in the literature	Craggs et al., (2012)
	7.01	kg(DW).d-1	Calculated conisdering removal efficiency following coagulation	
	2,557.04	kg(DW).yr-1		
	1.97			

DAF harvesting

	Value	Unit	Notes	Reference
DAF energy demand	0.30	kWh.m-3	Cost+Assumptions sheet	Molina Grima et al., (2003)
	3,942.00	**kWh.yr-1**		
Overflow solids concentration	3.00	%TS		Rawat et al., (2013)
DAF CAPEX	**175,046.34**	**£**		

Methane/energy production

	Value	Unit	Notes	Reference
VS content	0.90	%	Cost+Assumptions sheet	Ometto et al., (2014)
	6.31	kgVS.d-1	Calculated	
CH4 production	0.48	m3.kg VS-1	Cost+Assumptions sheet, assuming enzymatic pretreatment	Ometto et al., (2014)
Digestion effiency	0.80	%	Cost+Assumptions sheet	Ometto et al., (2014)
Total CH4 production	2.42	m3 methane.d-1	Calculated	
Engine generator efficiency	0.30	%	Cost+Assumptions sheet	Ometto et al., (2014)
	9.70	kWh.m-3 methane	Cost+Assumptions sheet	Ometto et al., (2014)
Energy production	7.05	kWh.d-1	Calculated	
Energy generated	**2,571.61**	**kWh.yr-1**	Calculated	

Pump cost	**55,433.82**	**£**	Storage tank, transfer pumps, metering pumps, piping, valves and facility enclosure. Based on liquid Al feed.	McGivney, W.T. (2008), pg 39

E.2.5 Scenario D: Immobilised bioreactor (IBR)

OVERALL SUMMARY

	No. of reactors			
	2.00	**3.00**	**4.00**	**5.00**
OPEX				
Bead cost.yr-1	5,226.48	5,226.48	5,226.48	5,226.48
kWh.yr-1	227,074.70	226,405.07	226,070.26	225,869.37
Elec £.yr-1	19,301.35	19,244.43	19,215.97	19,198.90
Sub-total (£.yr-1)	24,527.83	24,470.91	24,442.45	24,425.38
kWh.yr-1 (generated)	-103,516.76	-103,516.76	-103,516.76	-103,516.76
Elec £.yr-1 (generated)	-8,798.92	-8,798.92	-8,798.92	-8,798.92
Total spend £.yr-1	24,527.83	24,470.91	24,442.45	24,425.38
CAPEX (£)	474,318.53	474,978.57	475,793.55	476,958.63
Fixed Capital	**1,185,796.32**	**1,187,446.44**	**1,189,483.89**	**1,192,396.57**

ASSUMPTIONS/OMISSIONS

Operation cycle, inlcuding 1 day to fill and 1 day to empty.

DESIGN CALCULATIONS

Sizing

	Value	**Unit**	**Notes**	**Reference**
P influent	1.00	mg.L-1		
Influent	360.00	m3.d-1	2,000 PE	
	360,000.00	L.d-1		
Req. HRT	1.90	h	**Can be manipulated**	
	0.08	d		
P load	28,500.00	mg		
	28,500,000.00	ug		
Total P loading and bead uptake rate		y = 45.433 ln^-3.196x	Relationship obsereved for P loading and bead uptake rate	Thesis, Chapter 4
Batch run time (y)	16.55	days	**Can be manipulated**	
Bead uptake rate (x)	0.32	ug.beads-1.d-1	Calculated	
Bead no	1.14E+09	un	Calculated	
Bead diameter (approx)	2.97	mm		Thesis, Chapter 4 & 5
	2.97E-03	m		
Bead radius	1.48E-03	m		
Bead volume (total)	15.58	m3	Calculated	
	15,581.26	L	Calculated	
Influent volume	28,500.00	L		
Beads.mL-1	39.98	un	**Must be below 40 beads.mL-1**	Thesis, Chaper 3
Bead + influent vol	44,081.26	L	Calculated	
	44.08	m3		

SINGLE MODULE

	Value	Unit	Notes	Reference
Effluent to be treated at chosen HRT	28.50	m3		
Volume of beads req.	15.58	m3 beads		
Total reactor volume	44.08	m3		
Reactor height (total)	3.00	m	M&E, UASB guidelines - 5 - 7 m	
'Head board' height	1.94	m	M&E, UASB guidelines - 1.5 - 2 m	
	TRUE		True - though should be roughly in range.	
Reactor area	14.69	m2		
Radius	2.16	m		
Diameter	4.33	m	Industry spec <5 m?	
Bead expansion height with fluidisation	30.00	%		
	0.32	m		
True 'head board' height'	1.62	m		
Headboard height as percentage of whole reactor	54.05	%		
Bead height	1.38	m		
Bead volume	20.26	m3		
Total reactor volume	44.08	m3		

MULTIPLE MODULES

No.	**2.00**	**3.00**	**4.00**	**5.00**
Volume (m3)	22.04	14.69	11.02	8.82
Area (m2)	7.35	4.90	3.67	2.94
Radius (m)	1.53	1.25	1.08	0.97
Diameter (m)	3.06	2.50	2.16	1.93
	TRUE	TRUE	TRUE	TRUE
Bead volume (m3)	7.79	5.19	3.90	3.12
Bead bed height (m)	1.06	1.06	1.06	1.06
True bead height with fluidisation	1.38	1.38	1.38	1.38
Flow (m3.h-1)	7.50	5.00	3.75	3.00
v (considering influent feed from works) (m/h)	1.02	1.02	1.02	1.02
Req. v at chosen HRT (bead contact time)	0.56	0.56	0.56	0.56
Recirculation within module	1.83	1.83	1.83	1.83
Volume of LED illumination	**1.37E-04**	**m**		
No of LEDS	**53,525.93**	**35,683.95**	**26,762.96**	**21,410.37**

Required minimum fluidisation velocity

Bead diameter	2.97	mm
	0.002967	mm
Bead density	1,025.64	kg.m-3
Porosity	0.34	
Min Vmf	0.000733	m.s-1
	2.64	m.h-1

No.	**2.00**	**3.00**	**4.00**	**5.00**
Sufficient fludisation velocity with influent feed velocity	FALSE	FALSE	FALSE	FALSE

Recirculation pumps required

Footprint

SINGLE MODULE

	Value	**Unit**
Area	14.69	m2
Area with spacing	26.65	m2
	0.01	m2/PE
Spacing between reactors	1.50	m

Notes	**Reference**
Calculated	
Calculated	
Calculated	
Cost+Assumptions sheet	

MULTIPLE MODULES

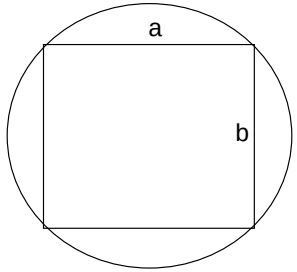
No.	**2.00**	**3.00**	**4.00**	**5.00**
Area per reactor (m2)	7.35	4.90	3.67	2.94
Area per reactor with spacing (per reactor)	16.32	12.55	10.54	9.26
Total footprint (all reactors) m2	32.64	37.65	42.15	46.32
Bead reservoir tanks (new + spent)	51.10	51.10	51.10	51.10
Total footprint (reactors + reservoirs) m2	83.74	88.74	93.24	97.42
Enough land available?	TRUE	TRUE	TRUE	TRUE
m2/PE	0.04	0.04	0.05	0.05

Civils
SINGLE MODULE

	Value	Unit	Notes	Reference
Excavation depth	0.60		Cost+Assumptions sheet	
Excavation	15.99	m3	Calculated	
Cost	55.79	£	Info from cost sheet	
Land prep	26.65	m2		
Cost	5.70	£	Info from cost sheet	
Concrete plynth	1,488.04	£	Info from cost sheet	
Sub-total	**1,549.54**	**£**		

MULTIPLE MODULES

No.	**2.00**	**3.00**	**4.00**	**5.00**
Total footrpint (all reactors) m2	32.64	37.65	42.15	46.32
Excavation (m3)	19.58	22.59	25.29	27.79
Cost (£)	68.33	78.81	88.23	96.96
Land prep cost (£)	6.99	8.06	9.02	9.91
Concrete cost (£)	1,822.39	2,101.90	2,353.02	2,586.01
Sub total (£)	**1,897.70**	**2,188.77**	**2,450.27**	**2,692.89**



Lighting
SINGLE MODULE

	Value	Unit	Notes	Reference
Diameter	4.33	m		
Radius	2.16	m		
Length/width of square (a, b)	3.06	m		
Area of light square	9.35	m2		
Area % of total reactor illuminated	0.64	%		
Remaining unlit (dead space)	5.34	m2		
Each segment (dead space)	1.33	m2		
Percentage unlit space	36.34	%		
Light spacing	0.03200	m	Based on packed bed, 400 umol.m-2.s-1	Thesis, Chapter 5
Bulbs req. length (a)	95.58	un		
Bulbs req. width (b)	95.58	un		
Bulbs req. depth	43.08	un		
Total bulbs	393,528.40	un		
LED bulb viewing angles	120.00	degrees		
Total bulbs	1,180,585.20	un		

MULTIPLE MODULE

No.	**2.00**	**3.00**	**4.00**	**5.00**
Diameter	3.06	2.50	2.16	1.93
Radius	1.53	1.25	1.08	0.97
Length/width of square (a, b)	2.16	1.77	1.53	1.37
Area of light square	4.68	3.12	2.34	1.87
Area % of total reactor illuminated	0.64	0.64	0.64	0.64
Remaining unlit (dead space)	2.67	1.78	1.33	1.07
Each segment (dead space)	0.67	0.44	0.33	0.27
Bulbs req. length (a)	67.58	55.18	47.79	42.74
Bulbs req. width (b)	67.58	55.18	47.79	42.74
Bulbs req. depth	33.14	33.14	33.14	33.14
Total bulbs (with viewing angle) per reactor	**53,525.93**	**35,683.95**	**26,762.96**	**21,410.37**
Bulb cost (£)	**319,014.52**	**319,014.52**	**319,014.52**	**319,014.52**
Bulbs (kWh.yr-1)	**225,065.82**	**225,065.82**	**225,065.82**	**225,065.82**

Module - CAPEX
SINGLE/MULTIPLE MODULES

	Value	**Unit**
Total reactor volume	44.08	m3
Reactor tank cost	**50,090.70**	£

Notes	**Reference**
Info from cost sheet	

OPERATION
MULTIPLE MODULES

Fill (d)	1.00	1.00	1.00	1.00
React (d)	16.55	16.55	16.55	16.55
Empty (d)	1.00	1.00	1.00	1.00
Total cycle time	18.55	18.55	18.55	18.55
Start a reactor every (d)	9.28	5.18	3.64	2.71
Bead batches/top up each year	39.35	59.03	78.71	98.38
Av per month for all reactors	3.28	4.92	6.56	8.20
Av per month per reactor	1.64	1.64	1.64	1.64

Tanker size	10,000.00	L		
	10.00	m3		
Monthly spent beads to remove m3 (no degradation considered)	25.55	25.55	25.55	25.55
No. of tankers per month (remove spent beads) (un)	2.55	2.55	2.55	2.55
No. of tankers per month to replace beads (no storage) (un)	2.55	2.55	2.55	2.55

Tanker visits per annum	3.00			
Bead reservoir tanks (visit per annum) (m3)	76.65	76.65	76.65	76.65
Tankers every x months for reservoir top up	7.66	7.66	7.66	7.66
Bead reservoir tanks spent beads (x months) (m3)	76.65	76.65	76.65	76.65

	Value	**Unit**
Bead reservoir tank depth	3.00	m
Surface area	25.55	m2
Width	5.00	m
Length	5.11	m
Bead reservoir surface area (new beads + spent)	51.10	m2

Notes	**Reference**
Assumption	

	Value	**Unit**
Screen	**92,246.07**	**£**

Notes	**Reference**
Cost+Assumption sheet	

Hydraulic pumping

Influent E = Q.p.g.h/(1000.n)

No.	**2.00**	**3.00**	**4.00**	**5.00**	**Notes**	**Reference**
Q (m3.s-1)	0.002	0.001	0.001	0.001		
p (kg.m-3)	998.00	998.00	998.00	998.00		
g	9.81	9.81	9.81	9.81		
h (m)	1.06	1.06	1.06	1.06		
n	0.50	0.50	0.50	0.50		
E (kW)	0.04	0.03	0.02	0.02		
kWh.yr-1	378.93	252.62	189.47	151.57	Influent pump for all modules	
kWh.yr-1	**378.93**	**252.62**	**189.47**	**151.57**	Influent pump	
£.yr-1	**32.21**	**21.47**	**16.10**	**12.88**		
Total	**3,689.84**	**3,874.33**	**4,151.07**	**4,612.30**		

No.	**2.00**	**3.00**	**4.00**	**5.00**	**Notes**	**Reference**
Q (m3.s-1)	0.0021	0.0014	0.0010	0.0008		
p (kg.m-3)	998.00	998.00	998.00	998.00		
g	9.81	9.81	9.81	9.81		
h (m)	0.00	0.00	0.00	0.00		
n	0.50	0.50	0.50	0.50		
E (kW)	0.04	0.03	0.02	0.02		
kWh.yr-1	**357.35**	**238.23**	**178.67**	**142.94**		
£.yr-1	**30.37**	**20.25**	**15.19**	**12.15**		
Pump cost £	**3,689.84**	**3,874.33**	**4,151.07**	**4,612.30**		

Recirculation pumps

12.92

No.	**2.00**	**3.00**	**4.00**	**5.00**	**Notes**	**Reference**
Q (m3.s-1)	0.005	0.004	0.003	0.002		
p (kg.m-3)	998.00	998.00	998.00	998.00		
g	9.81	9.81	9.81	9.81		
h (m)	1.38	1.38	1.38	1.38		
n	0.50	0.50	0.50	0.50		
E (kW)	0.15	0.10	0.07	0.06		
kWh.yr-1	1,272.60	848.40	636.30	509.04		
kWh.yr-1	**1,272.60**	**848.40**	**636.30**	**509.04**		
Pump cost (£)	**3,689.84**	**3,689.84**	**3,689.84**	**3,689.84**		

E.3 CAPEX and OPEX estimates

Notes

CPI (overall index) **128** Consumer Price Index 2015

CAPEX

			Conversion of data from the literature							
	Unit	**Value**	**Value**	**Unit**	**Date**	**Cost Index**	**Currency**		**Notes**	**Reference**
GENERAL										
Excavation	£.m-3	3.49	3.26	£.m-3	2011	119.6	-	-		CESMM3 (2011), pg 53, E3..1.1.01
Land preparation	£.m-2	0.21	0.20	£.m-2	2011	119.6	-	-		CESMM3 (2011), pg 53 E6.4.1.01
Concrete	£.m-3	93.05	78.88	£.m-3	2008	108.5	-	-	Standard mix one + water repellant additive	SPONS, Civil Engineering (2008), pg 51
A: COAG+SF										
Sand filter	£	1,008,913.46								D. Inman, 2016, pers. comm, 2nd February
Coag dosing system	£	55,379.85	88,744.97	$	2007	104.7	0.51	£.$-1	Storage tank, transfer pumps, metering pumps, piping, valves and facility enclosure. Based on liquid Al feed.	McGivney, W.T. (2008), pg 39
B: COAG+AW										
Coag dosing system	£	55,379.85	88,744.97	$	2007	104.7	0.51	£.$-1	Storage tank, transfer pumps, metering pumps, piping, valves and facility enclosure. Based on liquid Al feed.	McGivney, W.T. (2008), pg 39
Aerated wetland	£.PE-1	350.94								D. Inman, 2016, pers. comm, 2nd February
C: HRAP										
Paddlewheel	£	12,076.42	20,000.00	$	2014	128	0.60	£.$-1	1.74 kW motor per pond paddlewheel.	Rogers et al. (2014)
Liner	£.m-2	4.57	3.87	£.m-2	2008	108.5	-	-	Lake Liners: Landline Ltd or 'Alkorplan' geomembranes to prepared surfaces; all joints welded, (assume 1.0 m thick) - SUDS for Roads workbook	SPONS, External Works (2008), pg 270
HRAP influent pump	£	2,767.38	3,000.00	$	2002	95.4	0.69	£.$-1	Single and multi-stage centrifugal pump, equivalent of 360 m3.d-1	Loh et al., (2002), pg 30
HRAP effluent pump	£	2,767.38	3,000.00	$	2002	95.4	0.69	£.$-1	Single and multi-stage centrifugal pump, equivalent of 360 m3.d-1	Loh et al., (2002), pg 30
Coag dosing system	£	55,433.82	88,831.47	$	2007	104.7	0.51	£.$-1	Storage tank, transfer pumps, metering pumps, piping, valves and facility enclosure. Based on liquid Al feed.	McGivney, W.T. (2008), pg 39
DAF unit	£	175,046.34								D. Inman, 2016, pers. comm, 2nd February
D: IBR										
Carbon steel tank	£.m-3	1,136.33	870.00	£.m-3	2004	98	-	-		Sinnott, R.K. (2005), Vol 6, pg 259
LED lighting system	£.bulb-1	2.98	2.98	-	2014	128	-	-	Including all ancilliary equipment, for LED bulbs with a PFD of up to 1,000 lumens. (2.98)	iXscient Ltd, 2014 , pers. comm, 22nd October
Screen	£	92,246.07	100,000.00	$	2002	95.4	0.69	£.$-1	Plate and frame screen flow rate equivalent of 0.25 m3.min-1	Loh et al., (2002), pg 26
Centrifugal pump	£	3,689.84	4,000.00	$	2002	95.4	0.69	£.$-1	0.02 kW	Loh et al., (2002), pg 30
Centrifugal pump	£	3,874.33	4,200.00	$	2002	95.4	0.69	£.$-1	0.03 kW	Loh et al., (2002), pg 30
Centrifugal pump	£	4,151.07	4,500.00	$	2002	95.4	0.69	£.$-1	0.035 kW	Loh et al., (2002), pg 30
Centrifugal pump	£	4,612.30	5,000.00	$	2002	95.4	0.69	£.$-1	0.04 kW	Loh et al., (2002), pg 30

OPEX

	Unit	**Value**	**Value**	**Unit**	**Date**	**Cost Index**	**Currency**		**Notes**	**Reference**
Electricity	£.kWh	0.09	-	-	-	-	-	-		D. Inman, 2014, pers. comm, 2nd May
FeCl3	£.ton-1	290.00	290.00	-	2014	128	-	-		M. Pidou, 2016, pers. comm, 27th January
Calcium chloride	£.ton-1	2,073.00	-	-	2015	-	-	-		Brenntag UK Ltd, 2015 , pers. comm, 3rd July
Na-alginate	£.kg-1	33.04	-	-	2015	-	-	-		Brenntag UK Ltd, 2015 , pers. comm, 3rd July

E.4 Manufacturer bead production costs

Table A.5 Manufacturer bead production costs.

Manufacturer	Immobilised organism	kg.yr^{-1}	€.yr^{-1a}	€.kg^{-1}	£.kg^{-1b}	Equivalent cost (£.10^{6} beads^{-1})	Reference
Lentikat's Biotechnologies	Bacteria	27,400	351,500	12.83	10.57	0.26	Lentikat's Biotechnologies (2013)
		20,500	263,500	12.85	10.59	0.26	
	Enzyme	11,900	150,000	12.61	10.39	0.26	
		9,500	120,000	12.63	10.41	0.26	
		3,600	46,000	12.78	10.53	0.26	
	Yeast	2,700	34,500	12.78	10.53	0.26	
		61,500	790,000	12.85	10.59	0.26	
		41,000	526,500	12.84	10.58	0.26	

[a] Quoted 2013 cost
[b] Converted to 2015 cost using CPI.