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Harvesting of algae in municipal wastewater treatment by calcium phosphate precipitation mediated by photosynthesis, sodium hydroxide and lime

Phasey J^{1&2*}, Vandamme D³, Fallowfield HJ²

¹ Power and Water Corporation, Darwin, Northern Territory, Australia

² Health and Environment Group, School of the Environment, Faculty of Science and Engineering, Flinders University, GPO Box 2100, Adelaide, South Australia 5001

³ Laboratory for Aquatic Biology, KU Leuven Campus Kulak, Etienne Sabbelaan 53, 8500 Kortrijk, Belgium

*corresponding Author: *E-mail address:* Jordan.phasey@jpw.com.au, Tel.: +61 (8) 8924 5001

Abstract

The cultivation of microalgae within municipal and agricultural wastewater offers the opportunity to treat the water and partition nutrients into valuable biomass. Harvesting of those algae is a challenge, as conventional coagulants (iron and aluminum salts) contaminate algae and lock up phosphorus preventing beneficial reuse. We show that flocculation by calcium phosphate precipitation is capable of harvesting algae and phosphate from municipal wastewater in a form suitable for reuse. However, the initiation of flocculation by natural pH increase was infeasible. We harvested 93% of algal solids with an initial concentration of 113 mg VSS per L and 96% of total phosphate from a waste stabilization pond effluent by initiating flocculation with 129 mg-P/L of soluble phosphate and addition of 364 mg/L quicklime to achieve a pH of 10, at a calculated cost of \$0.05 USD per kL for alkali addition, which is similar to the chemical addition costs of conventional coagulation.

Keywords

Harvesting; wastewater treatment; microalgae; phosphorus recovery; coagulation; flocculation

1.0 Introduction

Microalgal biomass can have an areal productivity orders of magnitude above that of conventional agricultural crops (Wijffels and Barbosa 2010, Judd et al. 2015), and this has increased the global interest to cultivate microalgae for the production of e.g. biofuels, biogas, high-protein animal feed (Christenson and Sims 2012). However, cost-effective harvesting of microalgal biomass is still a challenge (Vandamme et al. 2013, Barros et al. 2015, Judd et al. 2015). Secondly, the sustainable supply of nutrients for large-scale microalgal cultivation is a problem, as it will compete with existing fertilizer utilization in agriculture (Markou et al. 2014). Global phosphorus reserves are finite, and improving the sustainable use and reuse of this element is critical (Cordell 2009).

Cultivation of microalgae using municipal and agricultural wastewater in high rate algal ponds (HRAPs) partitions nutrients into algal biomass, which can be recovered and reused (Benemann et al. 1980, Cromar and Fallowfield 1997, Matassa et al. 2015, Park and Craggs 2011). Unfortunately, the iron and aluminum salts regularly used by the water industry, as coagulant to remove algae and excess phosphorus from wastewaters, are toxic in concentrated form, contaminating the algae and preventing its use as feed, while aluminum in particular binds so strongly to phosphate as to be practically irreversible, preventing its use as fertilizer (Bashan and Bashan 2004, Huang et al 2007). Some studies have explored the use of non-toxic flocculants such as cationic starch and chitosan on laboratory cultures, however they are currently either too expensive or require further development to become commercially viable in the framework of wastewater treatment (Vandamme et al. 2010; Show et al. 2013). In addition, very little work has been performed aimed at recovering wastewater derived algae biomass using such flocculants at the pilot scale using real wastewater.

One potential method to separate algae and phosphorus from phosphate-rich wastewaters in a form suitable for reuse is flocculation by calcium phosphate precipitation. This phenomenon occurs when water containing dissolved calcium and orthophosphate ions experiences a rise in pH, e.g. by the photosynthetic activity of microalgae, resulting in the precipitation of amorphous calcium phosphate. These precipitates are thought to have a positive surface charge, and are electrostatically attracted to negatively charged algae cells (Sukenik et al. 1985). Electrostatic attraction leads to enmeshment of microalgal cells within the charged precipitates, increasing their density and causing them to settle, which has been described by Sukenik and Shelef (1984) as “autoflocculation”.

Flocculation by calcium phosphate precipitation has already been demonstrated in laboratory cultures of *Chlorella vulgaris* by Beuckels et al. (2013), and the presence of algogenic organic matter had a substantial inhibitory effect upon flocculation performance. This finding is important because e.g. municipal wastewater contains dissolved organic carbon (DOC), which is known to interfere with flocculation (Sukenik and Shelef 1984, Vandamme et al. 2012). However, charge density measurements may be an interesting control tool for water characterization with respect to coagulant dosage (Henderson et al. 2010).

This study aims to evaluate whether microalgae flocculation grown on municipal wastewater by calcium phosphate precipitation is achievable either naturally by algal photosynthetic pH increase, or using alkali such as sodium hydroxide or slaked lime. The conditions (dose, pH, calcium to phosphorus ratio) and the costs necessary to achieve effective flocculation followed by sedimentation were determined. Charge density measurements were conducted to explain the observed differences in required coagulant dosages between municipal wastewater and laboratory microalgal cultures.

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76 **2.0 Materials and Methods**

77 **2.1 Water Sample Collection and characterization**

78 Waste stabilisation pond (WSP) effluent was collected from the final outlet weir of
79 the Leanyer/Sanderson WwTP, which treats the municipal wastewater from the northern
80 suburbs of Darwin, Northern Territory, Australia; a population of 48,000 equivalent persons.
81 The pond system has two identical treatment trains, consisting of a facultative pond and four
82 maturation ponds. Sample collection always occurred in the morning prior to performing
83 experiments. The WSP effluent was analyzed for a range of physical and chemical
84 parameters by the Northern Territory Government Department of Primary Industries and
85 Fisheries Water Chemistry Laboratory in Darwin NT, Australia (Table 1): biochemical
86 oxygen demand (BOD₅ - APHA 5210B), chemical oxygen demand (COD - APHA 5220B),
87 suspended solids (SS - APHA 2540D), volatile suspended solids (VSS - APHA 2540E), Free
88 ammonia as N (NH₃-N - APHA 4500-NH₃ G), organic nitrogen as N (org-N - APHA 4500N-
89 org D), nitrate and nitrite as N (NO_x-N - APHA 4500-NO₃⁻ E), filterable reactive
90 orthophosphate as P (FR PO₄-P - APHA 4500P F), total phosphorus as P (TPO₄-P - APHA
91 4500P C), electrical conductivity (EC - APHA 2510B), pH (APHA 4500 H⁺ -B), total
92 alkalinity (APHA 2320B), bicarbonate alkalinity (HCO₃⁻ - APHA 2320B), carbonate
93 alkalinity (CO₃²⁻ - APHA 2320B), hydroxide alkalinity (OH⁻ - APHA 2320B), total hardness
94 (APHA 3120B), calcium (Ca - APHA 3120B) and magnesium (Mg - APHA 3120B).

Table 1. Chemical characterization of WSP effluent effluents (i) Day 1 – pH increase by photosynthesis without addition of alkali, and pH increased by NaOH addition; (ii) Day 36 – used for investigation of flocculation induced by slaked lime; (iii) Day 49 – used for charge density measurements

WSP Sample date	NaOH (Day 1)	Ca(OH) ₂ (Day 36)	Charge Density (Day 49)
BOD ₅ (mg/L)	62	57	74
COD (mg/L)	250	200	300
SS (mg/L)	110	125	175
VSS (mg/L)	102	113	150
Free NH ₃ -N (mg/L)	15	1.3	1
Org -N (mg/L)	17	17	18
NO _x -N (mg/L)	<0.1	<0.1	<0.1
FR PO ₄ -P (mg/L)	2.8	3.3	3.2
TPO ₄ -P (mg/L)	6.4	6.9	6.6
EC (µS/cm)	630	602	600
pH	7.74	7.59	8.01
Alkalinity (mg CaCO ₃ /L)	150	120	120
HCO ₃ (mg/L)	190	140	150
CO ₃ (mg/L)	<10	<10	<10
OH (mg/L)	<1	<1	<1
Total Hardness (mg CaCO ₃ /L)	42.2	53.8	51.3
Ca (mg/L)	7	10	9
Mg (mg/L)	6	7	7

2.2 Flocculation by calcium phosphate precipitation using photosynthesis, sodium hydroxide or slaked lime

Flocculation-sedimentation of algae in municipal wastewater effluent by calcium phosphate precipitation was evaluated based on flocculation jar testing (Phipps and Bird PB-900 jar tester). Various concentrations of calcium phosphate were tested through addition of pre-prepared calcium phosphate stock solutions to the native dissolved calcium and phosphate present in the WSP effluent (Table 1). The calcium phosphate stock solutions were prepared by mixing disodium hydrogen phosphate (Na₂HPO₄) and calcium chloride (CaCl₂)

in MilliQ water at various calcium to phosphorus (Ca:P) molar ratios (1.33:1, 1.5:1 and 1.67:1). These stock solutions were acidified using concentrated hydrogen chloride (37%) until all formed precipitates were dissolved. After addition of calcium phosphate, pH was increase using three different methodologies: (1) pH increase by photosynthesis without addition of alkali (Table 1, day 1); (2) addition of 0.1 M sodium hydroxide (Table 1, day 1); and (3) addition of 1.8 M slaked lime (Table 1, day 36). For the addition of slaked lime, 100 g of commercially available lime (CaO, 88% w/w) was ‘slaked’ with 1 L MilliQ water. pH was adjusted and monitored at four different levels (pH 7-8-9-10).

Flocculation was assessed based on a standardized jar test protocol (Bratby 2016). 1L of municipal wastewater sample was first mixed rapidly at 100 rpm during chemical addition, followed additionally by 5 more minutes of rapid mixing. This was followed by 30 mins of slow mixing at 15 rpm to allow floc growth during flocculation, followed by 30 mins of settling time. After settling, a sample was extracted, consistently 2 cm below the surface from each jar test to determine the separation efficiency based on turbidity analysis.

The separation efficiency was defined as the percentage reduction in turbidity before and after flocculation-sedimentation using Equation 1 (Gutiérrez et al. 2015; Hansel et al. 2014; Vandamme et al. 2012):

$$E_f = ((T_i - T_f) / T_i) \times 100 \quad \text{Equation 1}$$

Where E_f = Separation efficiency (%), T_i = initial turbidity (NTU) and T_f = final turbidity (NTU). Prior to chemical addition, T_i of each jar was determined using a Hach H2100Q handheld turbidity meter.

The flocculated supernatant was analyzed for a range of physical and chemical parameters by the Northern Territory Government Department of Primary Industries and

Fisheries Water Chemistry Laboratory in Darwin NT, Australia: BOD₅, NH₃-N, org-N FR PO₄-P, TPO₄-P and SS. Due to limited sample volume, the clarified effluent from the triplicate jar tests was decanted from the settled floc and combined for water quality characterization.

2.3 Charge Density Analysis

The method of charge density measurement was adapted from the methods of Henderson et al. (2010) and Kam and Gregory (1999) using a Mutek PCD-05 (BTG Pty Ltd Melbourne) streaming current detector with auto titrator and 0.001N polydiallyldimethylammonium chloride (Polydadmac). The Polydadmac was purchased in standardized solution from BTG Pty Ltd (Melbourne) and standardized with 0.001N polyethylene sulfonate (PES; BTG Pty Ltd Melbourne). Throughout the study, both Polydadmac and PES polyelectrolytes were regularly tested for degradation of charge equivalences, no degradation was detected. The required pH of the effluent was adjusted using either 0.1 M NaOH or 0.1 M HCl and was buffered using 0.1 M Tris(hydroxymethyl)aminomethane (TRIS) buffer for pH 7 and pH 8, and 0.1 M NH₄Cl for pH 9 and 10. The charge density of the effluent at each pH was determined through three colloid titrations, using 40, 50 and 60 mL of effluent respectively. Linear regression was performed, with the slope of the line at each pH taken to be the charge density of the water in equivalents per volume.

2.4 The Relative Charge Density of the Solid and Dissolved Constituents of WSP Effluent

In order to determine the relationship between suspended solids content and charge density (pH 7), the WSP effluent (Suppl. Table 1, day 49) was modified as follows. A portion of the WSP effluent was centrifuged for 15 minutes at 1,500 x g using a Sorvall RC-3B

centrifuge and analyzed as described above. A portion of the supernatant was also filtered through a 0.45 µm filter, and analyzed as described in section 2.3. Also, dissolved organic carbon content (DOC – APHA 5310 D) was determined by the Australian Water Quality Centre (Adelaide, South Australia).

To achieve an increasing concentration of suspended solids within the WSP effluent, while retaining the same concentration of dissolved constituents (DOC, extracellular organic matter etc.), a portion of WSP effluent was centrifuged, the solids collected and varying amounts re-suspended in three separate samples of untreated WSP effluent. The charge density (pH 7) and suspended solids of these samples was determined. Linear regression was performed to define the relationship between the charge density (pH 7) and suspended solids content of the samples. A one-way ANOVA (SPSS Statistics software) was performed to determine the statistical significance of the relationship. The charge density of the wastewater derived DOC was calculated using Equation 2:

$$C_{\text{DOC}} = C_{\text{DIS}} / [\text{DOC}] \quad \text{Equation 2}$$

Where C_{DOC} is the charge density of the dissolved organic carbon, in mEq/g Carbon; C_{DIS} is the charge density of the dissolved fraction of the wastewater, taken as the y intercept of the linear regression relationship depicted in Figure 3; and $[\text{DOC}]$ is the concentration of dissolved organic carbon in the water in mg of C per L.

2.5 Water chemistry modeling and cost calculations

Visual MINTEQ was used to explore the effect of photosynthetic inorganic carbon uptake on the precipitation of calcium phosphate. Two Scenarios were modelled, a control Scenario 1, based on the WSP effluent composition on Day 1 (Table 1), and Scenario 2 based on the same WSP effluent composition but with additional soluble calcium and phosphate

present (22 mg/L PO₄-P; 1.33:1 Ca:PO₄). In both Scenarios the initial pH of water in the model was adjusted (to the nearest 0.5 pH unit) through addition of acid such that hydroxyapatite was dissolved. This was done as it is considered that the precipitation of calcium phosphate is a critical part of the mechanism, and should be dissolved prior to initiation of flocculation. The final pH at the end of the titration, once all the inorganic carbon had been removed was recorded to determine whether photosynthetic inorganic carbon uptake could induce flocculation by calcium phosphate precipitation. Modelled titration results were verified using identical Scenarios in PHREEQC. Visual MINTEQ was also used to estimate the dosage of Ca(OH)₂ required to achieve flocculation conditions in each jar test through theoretical titration.

The water quality characterization of the WSP effluent on Day 1 (Table 1) was used as a basis for the cost calculations, with additional Ca and PO₄ added based upon the known amount added to each jar test. The lime was assumed to have a CaO content of 88% w/w. The cost of lime was assumed to be \$116 US /T (USGS 2015).

3.0 Results and Discussion

3.1 Water sample characterization

The water being treated was typical of WSP effluent and did contain a large number of suspended solids (110-175 mg/L; Table 1). Nutrient levels were normal, though the free ammonia content varied substantially. The majority of the nitrogen was present as organic nitrogen, presumably bound up within the algae present in the effluent. Approximately half the total phosphorus was present in the dissolved phase of the water. The water was notably soft, with low hardness, calcium and magnesium and low alkalinity due to Darwin's water being drawn from a dam.

The algal species composition, abundance and chlorophyll-a content of the WSP effluent was determined by the Australian Water Quality Centre in Adelaide, South Australia. Samples were received for analysis within 48 hours of collection. The effluent was dominated by cyanobacteria (Suppl. Table 1 and Suppl. Figure 1), with *Merismopedia sp.* the most abundant in terms of cells/mL followed by *Planktothrix sp.* The most abundant green alga present was *Chlorella sp.* (259,000 cells/mL). Chlorophyll-a content increased from 487 to 1,230 µg/L over time. The domination of the effluent by cyanobacteria was unusual when compared with other WSP effluents (Pearson 2005), though is common for this facility.

3.2 Flocculation initiated by photosynthesis versus using sodium hydroxide

Figure 1: Separation efficiency of WSP effluent by calcium phosphate precipitation using sodium hydroxide (NaOH) at various calcium to phosphorus ratios (Ca:P), to raise the pH and induce precipitation – error bars indicate one standard deviation from the mean (n=3).

The purpose of investigation using NaOH was to determine the dosage of dissolved calcium and phosphate necessary to achieve effective separation by flocculation. Flocculation was achieved, with the separation efficiency steadily increasing to a maximum of approximately 90% at a dissolved phosphate dose of 114 mg PO₄-P /L or 1.1 mg PO₄-P per mg VSS and a molar Ca : PO₄-P ratio of 1.67:1 (Figure 1). To explore the effect of photosynthesis upon flocculation, WSP effluent containing the identical dissolved calcium and phosphate concentrations as used in Figure 1(Ca:P ratio 1.67:1) were placed in the sun for 300 minutes and slowly mixed. A strong pH rise was observed in the control incubation

(final pH >10), but this was not observed in the incubations containing additional calcium and phosphate. Secondly, dissolved oxygen levels in all incubations was >150% saturation and oxygen micro-bubbles were visually observed (results not shown). These observations indicated photosynthetic activity. However, there was poor to no flocculation observed in all these incubations.

Chemical equilibrium modelling using Visual MINTEQ was performed in order to study the cause of the failure of flocculation to take place in the presence of algal photosynthesis (Table 2). The control Scenario 1 predicted a final equilibrium pH of 10.6, which corresponded with the made observations. Scenario 2, which comprised of WSP effluent additionally amended with 22 mg/L of dissolved PO₄-P (7.03 x 10⁻⁴ M PO₄) was predicted to reach a final pH of only 5.8.

Table 2: Input conditions, initial and predicted final pH values modelled from the removal of inorganic carbon from solution, as a surrogate for photosynthetic inorganic carbon uptake, using Visual MINTEQ in two scenarios: (1) based on WSP effluent composition at day 1 and (2) based on WSP effluent composition at day 1 + 22 mg/L PO₄-P (1.33:1 Ca:PO₄)

¹ Components (M)	Scenario 1 WSP day 1	Scenario 2
		WSP day 1 + 22 mg/L PO ₄ -P
Ca ⁺²	1.50 x 10 ⁻⁴	1.05 x 10 ⁻³
CO ₃ ⁻²	3.17 x 10 ⁻³	3.17 x 10 ⁻³
H ⁺¹	4.49 x 10 ⁻³	7.31 x 10 ⁻³
Mg ⁺²	2.47 x 10 ⁻⁴	2.47 x 10 ⁻⁴
Na ⁺¹	6.99 x 10 ⁻⁵	N/A
NH ₄ ⁺¹	1.07 x 10 ⁻³	1.07 x 10 ⁻³
PO ₄ ⁻³	2.95 x 10 ⁻⁵	7.03 x 10 ⁻⁴

Cl ⁻¹	N/A	2.52 x 10 ⁻³
Initial pH	6.5	5.5
Predicted max pH	10.6	5.8

¹ Components in Visual MINTEQ described refer to the total concentration in solution, ignoring the influence of pH on speciation.

It was considered that the addition of acid, necessary to ensure dissolution of the calcium phosphate prior to initiation of photosynthesis, prevented a pH rise through its effect on the carbonate buffering system. Below a pH of 6.3, carbonic acid (H₂CO₃) is the dominant form of inorganic carbon, and the removal of it does not significantly influence the hydrogen ion concentration of the water, preventing a strong rise in pH and a consequent precipitation of amorphous calcium phosphate (Bratby 2016). Practically the initial addition of acid to ensure dissolution of the calcium phosphate before addition to the WSP effluent is problematic since flocculation depends on a subsequent alkaline pH being achieved to cause precipitation of calcium phosphate. The high dosages of dissolved calcium and phosphate observed here to achieve flocculation suggest further addition of calcium and phosphate is necessary, and that acidification and recycling of the calcium phosphate is probably the most economical method of doing it. The modelled lack of a significant pH rise was important, as it demonstrated that uptake of inorganic carbon by algae was unlikely to lead to sufficient precipitation of calcium phosphate crystals, and hence lead to flocculation. As a result, it is considered that photosynthesis in this particular case alone was unlikely to be able to achieve the required rise in pH and that the addition of a chemical alkali was necessary in order to induce flocculation.

3.3 Flocculation Initiated by Lime Addition

Lime was used to initiate flocculation by calcium phosphate precipitation, and yielded good flocculation as shown in Figure 2. Separation efficiencies higher than 95% were achieved at a dissolved phosphate dosage of 129 mg/L PO₄-P or 1.1 mg PO₄-P per mg VSS at pH 8, 9 and 10, with > 90% separation efficiency achieved at dosage of 87 mg/L PO₄-P or 0.8 mg PO₄-P per mg of VSS at pH 8, 9 and 10 (p<0.001). But even at pH < 8 separation efficiency of 80% was achieved using 129 mg/L PO₄-P. Sukenik and Shelef (1984) reported a critical flocculation pH of 7.2 for flocculation of *Chlorella vulgaris* laboratory cultures at initial calcium concentration of 2.5mM (100 mg/L) Ca and 0.95mM or 29 mg/L of phosphorus.

Brady et al. 2014 have proposed a general coordination chemistry model for algal autoflocculation including for flocculation mediated by the formation of amorphous calcium phosphate precipitates. Calcium ions show affinity for the charged surface of algal cells which results in a higher local calcium concentration within the double layer surrounding algal cells than in the surrounding water. When supersaturation of calcium phosphate occurs, phosphate ions will collide with calcium ions in close proximity to the algal cells and lead to crystal nucleation very close to the algal surface. This precipitation formation causes enmeshment of the algal cells within the charged amorphous precipitates, which is also referred to as sweeping flocculation (Sukenik et al. 1985, Beukels et al. 2013). At higher pH levels, precipitation of magnesium hydroxide and calcite can additionally play a role in the flocculation mechanism based on a combination of charge neutralization and sweeping flocculation (Garcia-Pérez et al. 2014).

The composition of the effluent after flocculation is shown in Table 3. Generally, higher initial dissolved calcium and dissolved phosphate doses and higher flocculation pH contributed to higher effluent quality. Importantly, phosphate removal was outstanding, with the 129 mg/L PO₄-P addition at pH of 10 yielding a total phosphate content in the effluent of

0.28 mg PO₄-P /L after treatment. This combination of pH and phosphate harvested 93% of the algal solids with an initial concentration of 113 mg VSS per L and 96% of the total phosphate. This phosphate is both contained within the algal biomass, and external to it as precipitated amorphous calcium phosphate. This means that both forms are suitable for phosphorus recovery and reuse in various applications.

Figure 2: Separation efficiency of WSP effluent by calcium phosphate precipitation using lime (Ca(OH)₂) to raise the pH and induce precipitation – error bars indicate one standard deviation from the mean (n=3).

The use of lime to initiate flocculation did not materially reduce the dosage of dissolved phosphate necessary to achieve flocculation (compared to the use of NaOH), with the optimum treatment identified in this study to be a dissolved phosphate dosage of 129

mg/L PO₄-P and a flocculation pH of 10. This high dose and high pH consequently resulted in higher doses of lime to achieve the desired pH. Visual MINTEQ modelling estimated that a dosage of 362 mg/L (6.5 mM CaO) quicklime would be required for the above-mentioned Scenario, at a cost of \$0.05 USD per kL. This cost was compared with the estimated cost of chemical addition at some Australian wastewater treatment plants (WwTPs), shown in Table 4. The cost of lime addition in this study was comparable with some and even much less than others, which is an encouraging outcome for those interested in developing sustainable strategies to harvest algae and recover phosphorus.

Table 3 Characterization of WSP effluent and percent reduction in contaminant content after flocculation by calcium phosphate precipitation using Ca(OH)₂ to raise pH and induce precipitation

Initial pH	PO ₄ -P (mg/L)		BOD ₅	NH ₃ -N	Org N	FRPO ₄ -P	TPO ₄ -P	SS
7	129	mg/L	4.9	1.1	3.6	13	24	120
		%	91	15	79	-	-	4
8	66	mg/L	9.6	1	4.3	3.9	4.7	26
		%	83	23	75	-	32	79
	87	mg/L	6.0	1.0	2.9	3.2	3.6	12.0
		%	89	23	83	3	48	90
	129	mg/L	3.2	1.0	2.4	2.3	2.1	10.0
		%	94	23	86	30	70	92
9	66	mg/L	11	1.00	3.7	0.62	1.2	21.0
		%	81	23	78	81	83	83
	87	mg/L	6.0	1.0	3.0	0.49	0.88	11.0
		%	89	23	82	85	87	91
	129	mg/L	3.7	1.1	2.2	0.48	0.65	6.00
		%	94	15	87	85	91	95
10	66	mg/L	10	1.1	3.5	<0.1	0.58	14.0
		%	82	15	79	>97	92	89
	87	mg/L	7.0	1.0	2.8	<0.1	0.42	7.00
		%	88	23	84	>97	94	94

	129	mg/L %	2.9 95	1.1 15	2.2 87	<0.1 >97	0.28 96	12.0 90
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Table 4 Chemical cost calculation of flocculation by calcium phosphate using slaked lime for biomass recovery compared to existing coagulant processes applied in large, medium and small Australian Utilities

Utility	End use	Chemical used	Dosage	Cost of chemical ¹	Cost/kL	Total chemical cost/kL
This Study	N/A	CaO	354 mg/L	\$116/T ⁴	\$0.05	\$0.05
Large Australian Utility ³	Recycled water and aquifer storage and recovery	Aluminium Sulfate	160 mg/L	\$435/T ²	\$0.07	\$0.08
		Polymer	1.5 mg/L	\$3.70/kg	\$0.0056	
Medium Australian Utility ³	Discharge to river in Winter - PO ₄ target of 0.3 mg/L as P	Ferric sulfate	200 - 300 mLs/kL treated	\$0.52/L	\$0.10 - \$0.16	\$0.10 - \$0.16
Small Australian Utility ³	Unrestricted irrigation	Aluminium Chlorohydrate	45 mg/L	\$1,002/T	\$0.19	\$0.25
		Polymer	4 mg/L	\$6.17/kg	\$0.02	
		H ₂ SO ₄	62 mg/L	\$581/T	\$0.04	

¹ Costs are approximate - Converted from AUD to USD at exchange rate 0.74 AUD/USD

² Geddie P (2006) \$450 AUD/T in 2006, plus 3% per annum cpi increase, converted to USD

³ Data based on oral communication with various existing Australian Utilities

⁴ USGS (2015)

3.4 The Relative Charge Density of the Solid and Dissolved Constituents of WSP Effluent

The dose of required dissolved phosphate to achieve flocculation was at least ten times higher than those reported previously by Beuckels et al. (2013) in a laboratory study. Charge density measurements were performed in order to identify the cause for this significant difference, in the hope of gaining some insight into the practicality of algae harvesting by flocculation from treated wastewater. As demonstrated in Figure 3, it was found that the dissolved fraction of the WSP effluent contained 70% of the overall charge density, while the solids portion contained only 30%. The relationship was statistically significant ($p = 0.004$). The charge density of the dissolved portion is presumed to be due to the 16.9 mg/L as C of dissolved organic carbon (DOC). By calculation, the wastewater derived DOC was found to have a charge density of 4.1 meq/g DOC as C. The WSP effluent in this study was found to have a charge density 20-50 times higher than the charge density of the laboratory cultures published by Henderson et al. (2010). It is presumed that the reason for the very high dosages of dissolved phosphate to achieve flocculation was due to the very high charge density of the WSP effluent studied here, much higher than laboratory cultures. These findings suggest that the relative contribution of the dissolved phase of municipal wastewater (wastewater derived DOC, algogenic organic matter, extracellular polysaccharides etc.) to the coagulant demand is even more important than presumed before. These findings should elicit a note of caution, as they provide evidence that laboratory cultures are not always viable models to predict the coagulation behavior of algae cultivated in wastewater, particularly when estimating chemical dose requirements and therefore costs. Future research should therefore focus on the mechanism and impact of dissolved organic matter on coagulant demand and performance in different media.

Figure 3: Correlation between WSP effluent solids content and charge density. One replicate of each observation was performed.

Conclusions

Harvesting of algae from municipal wastewater by calcium phosphate precipitation was effective and it allowed phosphate recovery, but it could not be achieved in this study by solely depending upon algal photosynthesis to elevate pH. Flocculation induced by lime addition achieved good algal harvesting results and very high quality effluent, while the cost of alkali addition was competitive with conventional coagulants. However, high doses of calcium and phosphate were needed, which are generally much higher than reported in most laboratory studies to date. The charge density of WSP effluent solids suggest it is very different in nature than most laboratory cultures.

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453 **Supplementary Material**

454 **Suppl. Figure 1: Algal and microbial composition of the WSP effluent at day 1 (type**
455 **light microscope, enlargement)**

456

457

Suppl. Table 1. Algal species composition of the WSP effluents (i) Day 1 – used in the preliminary NaOH experiments; (ii) Day 36 – used for investigation of flocculation induced by lime and (iii) Day 49 - used for the measurement of relative charge density of solids and dissolved constituents of WSP effluent.

Cells/mL	NaOH Day 1	Ca(OH)₂ Day 36	Charge density exp. Day 49
Cyanobacteria			
<i>Planktothrix sp.</i>	209,000	1,470,000	1,590,000
<i>Arthrospira sp.</i>	10,000-100,000	246,000	362,000
<i>Microcystis flos-aquae</i> <i>sp.</i>	4480	515,000	801,000
<i>Merismopedia sp.</i>	1,250,000	12,800,000	5,970,000
<i>Romeria sp.</i>	N/O	100-1000	1,000-10,000
Diatoms			
<i>Nitzschia sp.</i>	N/O	N/O	10-1000
Euglenophytes			
<i>Euglena sp.</i>	100-1000	10-100	N/O
<i>Phacus sp.</i>	N/O	10-100	10-100
Chlorophyceae			
<i>Monoraphidium sp.</i>	41,300	13,100	5,900
<i>Scenedesmus sp.</i>	7,300	4,300	1,000-10,000
<i>Chlorella sp.</i>	295,000	1,000-10,000	1,000-10,000
Microbial filaments			
Chlorophyll a (µg/L)	487	844	1,230

N/O – none observed.

Supplementary Table 2: Raw data to support calculation of separation efficiency of WSP effluent using NaOH (Figure 1) and Ca(OH)₂ (Figure 2)

Type of base	pH	Ca:PO₄ ratio	PO₄ (mg/L as P)	Initial turbidity (NTU)	Final turbidity (NTU)	Reduction in turbidity (%)
NaOH	9.00	1.33	2.80	139.00	121.00	0.13
NaOH	9.00	1.33	2.80	131.00	119.00	0.09
NaOH	9.00	1.33	2.80	127.00	112.00	0.12
NaOH	9.00	1.33	23.67	130.00	75.50	0.42
NaOH	9.00	1.33	23.67	132.00	77.60	0.41
NaOH	9.00	1.33	23.67	120.00	69.00	0.43
NaOH	9.00	1.33	44.55	135.00	42.80	0.68
NaOH	9.00	1.33	44.55	135.00	45.60	0.66
NaOH	9.00	1.33	44.55	121.00	47.10	0.61
NaOH	9.00	1.33	65.42	132.00	31.80	0.76
NaOH	9.00	1.33	65.42	138.00	38.20	0.72
NaOH	9.00	1.33	65.42	119.00	38.80	0.67
NaOH	9.00	1.33	86.29	132.00	29.00	0.78

NaOH	9.00	1.33	86.29	128.00	30.60	0.76
NaOH	9.00	1.33	86.29	119.00	35.30	0.70
NaOH	9.00	1.33	128.04	133.00	14.80	0.89
NaOH	9.00	1.33	128.04	127.00	15.70	0.88
NaOH	9.00	1.33	128.04	123.00	19.20	0.84
NaOH	9.00	1.50	2.80	118.00	110.00	0.07
NaOH	9.00	1.50	2.80	116.00	110.00	0.05
NaOH	9.00	1.50	2.80	120.00	101.00	0.16
NaOH	9.00	1.50	22.69	117.00	70.00	0.40
NaOH	9.00	1.50	22.69	121.00	67.90	0.44
NaOH	9.00	1.50	22.69	120.00	62.50	0.48
NaOH	9.00	1.50	42.59	119.00	44.10	0.63
NaOH	9.00	1.50	42.59	122.00	45.30	0.63
NaOH	9.00	1.50	42.59	115.00	42.30	0.63
NaOH	9.00	1.50	62.48	116.00	37.80	0.67
NaOH	9.00	1.50	62.48	116.00	33.50	0.71
NaOH	9.00	1.50	62.48	114.00	35.90	0.69

NaOH	9.00	1.50	82.38	118.00	27.20	0.77
NaOH	9.00	1.50	82.38	122.00	29.90	0.75
NaOH	9.00	1.50	82.38	119.00	31.20	0.74
NaOH	9.00	1.50	122.17	116.00	27.30	0.76
NaOH	9.00	1.50	122.17	124.00	26.30	0.79
NaOH	9.00	1.50	122.17	108.00	26.10	0.76
NaOH	9.00	1.67	2.80	103.00	98.10	0.05
NaOH	9.00	1.67	2.80	107.00	95.20	0.11
NaOH	9.00	1.67	2.80	96.80	94.20	0.03
NaOH	9.00	1.67	21.39	106.00	61.80	0.42
NaOH	9.00	1.67	21.39	106.00	52.00	0.51
NaOH	9.00	1.67	21.39	100.00	53.50	0.47
NaOH	9.00	1.67	39.98	99.60	35.60	0.64
NaOH	9.00	1.67	39.98	102.00	30.50	0.70
NaOH	9.00	1.67	39.98	104.00	28.40	0.73
NaOH	9.00	1.67	58.57	102.00	24.70	0.76
NaOH	9.00	1.67	58.57	99.00	23.90	0.76

NaOH	9.00	1.67	58.57	104.00	23.00	0.78
NaOH	9.00	1.67	77.16	98.30	19.70	0.80
NaOH	9.00	1.67	77.16	100.00	16.70	0.83
NaOH	9.00	1.67	77.16	104.00	19.00	0.82
NaOH	9.00	1.67	114.34	99.80	6.64	0.93
NaOH	9.00	1.67	114.34	98.00	7.67	0.92
NaOH	9.00	1.67	114.34	99.80	7.45	0.93
NaOH	10.00	1.33	2.80	123.00	119.00	0.03
NaOH	10.00	1.33	2.80	124.00	109.00	0.12
NaOH	10.00	1.33	2.80	113.00	109.00	0.04
NaOH	10.00	1.33	23.67	123.00	67.10	0.45
NaOH	10.00	1.33	23.67	122.00	62.10	0.49
NaOH	10.00	1.33	23.67	118.00	56.70	0.52
NaOH	10.00	1.33	44.55	121.00	49.20	0.59
NaOH	10.00	1.33	44.55	120.00	39.70	0.67
NaOH	10.00	1.33	44.55	115.00	43.90	0.62
NaOH	10.00	1.33	65.42	125.00	40.50	0.68

NaOH	10.00	1.33	65.42	117.00	37.60	0.68
NaOH	10.00	1.33	65.42	120.00	35.40	0.71
NaOH	10.00	1.33	86.29	125.00	36.00	0.71
NaOH	10.00	1.33	86.29	120.00	36.40	0.70
NaOH	10.00	1.33	86.29	119.00	37.80	0.68
NaOH	10.00	1.33	128.04	127.00	26.70	0.79
NaOH	10.00	1.33	128.04	121.00	24.40	0.80
NaOH	10.00	1.33	128.04	118.00	26.80	0.77
NaOH	10.00	1.50	2.80	108.00	99.30	0.08
NaOH	10.00	1.50	2.80	113.00	98.80	0.13
NaOH	10.00	1.50	2.80	105.00	97.20	0.07
NaOH	10.00	1.50	22.69	109.00	60.90	0.44
NaOH	10.00	1.50	22.69	110.00	51.90	0.53
NaOH	10.00	1.50	22.69	103.00	58.50	0.43
NaOH	10.00	1.50	42.59	113.00	38.70	0.66
NaOH	10.00	1.50	42.59	110.00	38.90	0.65
NaOH	10.00	1.50	42.59	105.00	32.70	0.69

NaOH	10.00	1.50	62.48	114.00	29.80	0.74
NaOH	10.00	1.50	62.48	110.00	36.60	0.67
NaOH	10.00	1.50	62.48	102.00	27.40	0.73
NaOH	10.00	1.50	82.38	107.00	33.30	0.69
NaOH	10.00	1.50	82.38	107.00	28.20	0.74
NaOH	10.00	1.50	82.38	108.00	28.60	0.74
NaOH	10.00	1.50	122.17	108.00	28.30	0.74
NaOH	10.00	1.50	122.17	113.00	25.90	0.77
NaOH	10.00	1.50	122.17	100.00	23.50	0.77
NaOH	10.00	1.67	2.80	108.00	95.20	0.12
NaOH	10.00	1.67	2.80	105.00	93.30	0.11
NaOH	10.00	1.67	2.80	98.10	93.10	0.05
NaOH	10.00	1.67	21.39	105.00	54.30	0.48
NaOH	10.00	1.67	21.39	102.00	46.70	0.54
NaOH	10.00	1.67	21.39	99.70	45.00	0.55
NaOH	10.00	1.67	39.98	111.00	28.80	0.74
NaOH	10.00	1.67	39.98	101.00	29.00	0.71

NaOH	10.00	1.67	39.98	102.00	30.90	0.70
NaOH	10.00	1.67	58.57	108.00	23.10	0.79
NaOH	10.00	1.67	58.57	99.80	24.40	0.76
NaOH	10.00	1.67	58.57	103.00	24.30	0.76
NaOH	10.00	1.67	77.16	107.00	22.20	0.79
NaOH	10.00	1.67	77.16	102.00	21.00	0.79
NaOH	10.00	1.67	77.16	100.00	19.80	0.80
NaOH	10.00	1.67	114.34	105.00	8.30	0.92
NaOH	10.00	1.67	114.34	68.70	11.10	0.84
NaOH	10.00	1.67	114.34	98.20	12.10	0.88
Ca(OH)2	7.00	N/A	3.30	202.00	193.00	0.04
Ca(OH)2	7.00	N/A	3.30	190.00	212.00	-0.12
Ca(OH)2	7.00	N/A	3.30	216.00	199.00	0.08
Ca(OH)2	7.00	N/A	24.17	197.00	203.00	-0.03
Ca(OH)2	7.00	N/A	24.17	196.00	208.00	-0.06
Ca(OH)2	7.00	N/A	24.17	209.00	205.00	0.02
Ca(OH)2	7.00	N/A	45.05	199.00	166.00	0.17

Ca(OH) ₂	7.00	N/A	45.05	200.00	179.00	0.11
Ca(OH) ₂	7.00	N/A	45.05	201.00	182.00	0.09
Ca(OH) ₂	7.00	N/A	65.92	197.00	150.00	0.24
Ca(OH) ₂	7.00	N/A	65.92	195.00	149.00	0.24
Ca(OH) ₂	7.00	N/A	65.92	192.00	160.00	0.17
Ca(OH) ₂	7.00	N/A	86.79	197.00	69.80	0.65
Ca(OH) ₂	7.00	N/A	86.79	204.00	68.50	0.66
Ca(OH) ₂	7.00	N/A	86.79	215.00	90.10	0.58
Ca(OH) ₂	7.00	N/A	128.54	212.00	25.00	0.88
Ca(OH) ₂	7.00	N/A	128.54	196.00	26.90	0.86
Ca(OH) ₂	7.00	N/A	128.54	198.00	42.00	0.79
Ca(OH) ₂	8.00	N/A	3.30	196.00	204.00	-0.04
Ca(OH) ₂	8.00	N/A	3.30	200.00	198.00	0.01
Ca(OH) ₂	8.00	N/A	3.30	207.00	194.00	0.06
Ca(OH) ₂	8.00	N/A	24.17	211.00	153.00	0.27
Ca(OH) ₂	8.00	N/A	24.17	202.00	168.00	0.17
Ca(OH) ₂	8.00	N/A	24.17	196.00	161.00	0.18

Ca(OH) ₂	8.00	N/A	45.05	197.00	70.90	0.64
Ca(OH) ₂	8.00	N/A	45.05	197.00	72.80	0.63
Ca(OH) ₂	8.00	N/A	45.05	193.00	64.90	0.66
Ca(OH) ₂	8.00	N/A	65.92	191.00	35.90	0.81
Ca(OH) ₂	8.00	N/A	65.92	205.00	36.70	0.82
Ca(OH) ₂	8.00	N/A	65.92	196.00	24.60	0.87
Ca(OH) ₂	8.00	N/A	86.79	206.00	17.00	0.92
Ca(OH) ₂	8.00	N/A	86.79	199.00	13.60	0.93
Ca(OH) ₂	8.00	N/A	86.79	214.00	9.37	0.96
Ca(OH) ₂	8.00	N/A	128.54	192.00	6.89	0.96
Ca(OH) ₂	8.00	N/A	128.54	202.00	6.33	0.97
Ca(OH) ₂	8.00	N/A	128.54	193.00	6.20	0.97
Ca(OH) ₂	9.00	N/A	3.30	203.00	210.00	-0.03
Ca(OH) ₂	9.00	N/A	3.30	191.00	215.00	-0.13
Ca(OH) ₂	9.00	N/A	3.30	214.00	207.00	0.03
Ca(OH) ₂	9.00	N/A	24.17	199.00	124.00	0.38
Ca(OH) ₂	9.00	N/A	24.17	203.00	117.00	0.42

Ca(OH) ₂	9.00	N/A	24.17	194.00	108.00	0.44
Ca(OH) ₂	9.00	N/A	45.05	205.00	65.20	0.68
Ca(OH) ₂	9.00	N/A	45.05	193.00	59.50	0.69
Ca(OH) ₂	9.00	N/A	45.05	217.00	47.90	0.78
Ca(OH) ₂	9.00	N/A	65.92	192.00	35.90	0.81
Ca(OH) ₂	9.00	N/A	65.92	208.00	29.60	0.86
Ca(OH) ₂	9.00	N/A	65.92	201.00	25.80	0.87
Ca(OH) ₂	9.00	N/A	86.79	209.00	16.30	0.92
Ca(OH) ₂	9.00	N/A	86.79	213.00	13.60	0.94
Ca(OH) ₂	9.00	N/A	86.79	193.00	13.00	0.93
Ca(OH) ₂	9.00	N/A	128.54	204.00	6.81	0.97
Ca(OH) ₂	9.00	N/A	128.54	190.00	6.13	0.97
Ca(OH) ₂	9.00	N/A	128.54	202.00	4.33	0.98
Ca(OH) ₂	10.00	N/A	3.30	205.00	180.00	0.12
Ca(OH) ₂	10.00	N/A	3.30	202.00	175.00	0.13
Ca(OH) ₂	10.00	N/A	3.30	210.00	180.00	0.14
Ca(OH) ₂	10.00	N/A	24.17	209.00	93.40	0.55

Ca(OH) ₂	10.00	N/A	24.17	205.00	100.00	0.51
Ca(OH) ₂	10.00	N/A	24.17	208.00	102.00	0.51
Ca(OH) ₂	10.00	N/A	45.05	200.00	49.70	0.75
Ca(OH) ₂	10.00	N/A	45.05	195.00	48.90	0.75
Ca(OH) ₂	10.00	N/A	45.05	207.00	48.50	0.77
Ca(OH) ₂	10.00	N/A	65.92	204.00	30.00	0.85
Ca(OH) ₂	10.00	N/A	65.92	195.00	27.70	0.86
Ca(OH) ₂	10.00	N/A	65.92	199.00	24.00	0.88
Ca(OH) ₂	10.00	N/A	86.79	209.00	15.10	0.93
Ca(OH) ₂	10.00	N/A	86.79	196.00	17.10	0.91
Ca(OH) ₂	10.00	N/A	86.79	209.00	18.20	0.91
Ca(OH) ₂	10.00	N/A	128.54	204.00	5.70	0.97
Ca(OH) ₂	10.00	N/A	128.54	194.00	8.14	0.96
Ca(OH) ₂	10.00	N/A	128.54	204.00	8.60	0.96

N/A – result is not available