




Evaluating the performance of conventional DAF and PosiDAF processes for cyanobacteria separation at a pilot plant scale

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ABSTRACT

In this work, a commercially available water treatment polymer poly(*N,N*-diallyl-*N,N*-dimethylammonium chloride) (PDADMAC) and a hydrophobically modified polymer (HMP) designed to adhere to bubble surfaces were applied for the first time in the novel Posi-dissolved air flotation process (PosiDAF) that uses polymer-modified bubbles, at pilot-scale for the treatment of waste stabilisation pond samples rich in algae. It was found that PDADMAC in PosiDAF gave comparable removal to that achieved using conventional DAF at >95% cell separation. Furthermore, the float layer was more uniform and thicker with up to 8% solid contents compared to conventional DAF, which comprised discrete floc clusters with an average solid concentration of ~4.1%. In contrast to the use of PDADMAC, the application of the HMP did not achieve similarly good separation at pilot scale. It was hypothesised that this may be due to the micellisation of the HMP on the bubble surface, creating unstable bubbles that coalesced and prevented polymer–bubble–cell interactions, which are crucial for effective cell separation. On comparison of the costs of PosiDAF and conventional DAF, it was found that PosiDAF resulted in cost-savings of up to 74% due to low chemical consumption. In summary, PosiDAF reduced chemical cost and increased solid contents in the metal-free float.

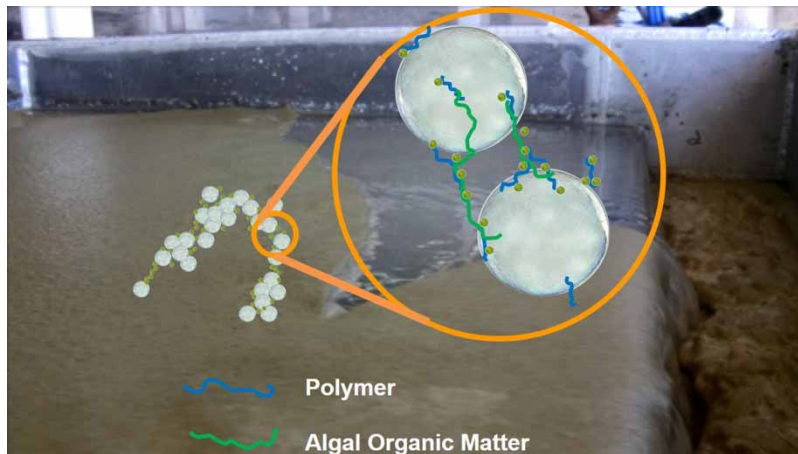
Key words: algae, biomass, flotation, harvesting, waste stabilisation pond

HIGHLIGHTS

- Effective translation of PosiDAF from laboratory to pilot-scale is achieved.
- PosiDAF float layer is structurally coherent compared to the conventional DAF float layer.
- Compared to conventional DAF, PosiDAF can reduce 74% costs due to low chemical use.

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GRAPHICAL ABSTRACT



1. INTRODUCTION

The presence of algal and cyanobacterial blooms in free surface wetlands and wastewater treatment plants, particularly in waste stabilisation ponds (WSPs), is commonplace, and therefore, there is interest in downstream cell separation to ensure the good quality effluent and potential uses including feedstock for digestion and nutrient recovery (Parmar *et al.* 2011). Of the methods explored for cell separation, dissolved air flotation (DAF) is considered to be a more effective and efficient clarification process than sedimentation for algae-laden waters due to the natural buoyancy of algae (Teixeira & Rosa 2006; Edzwald 2010; Granados *et al.* 2012; Vandamme *et al.* 2013). However, conventional DAF is highly dependent on effective coagulation–flocculation, where negatively charged particles are modified by the addition of a positively charged coagulant (Duan & Gregory 2003). Coagulation–flocculation can be challenging when the influent water character fluctuates rapidly such as during an algal bloom, either decreasing the separation efficiency or resulting in the use of excess coagulant (Edzwald 2010). Hence, chemical costs when applying coagulation–flocculation followed by DAF can fluctuate between 30 and 70% of the total process costs (Vandamme *et al.* 2013; Coons *et al.* 2014; Allnut & Kessler 2015; Dunlap *et al.* 2015; Demirbas 2017).

Re-engineering DAF through the use of polymers that coat and functionalise bubbles to generate positively charged bubbles that are attractive to negatively charged particles (termed PosiDAF in this paper) has shown promise at laboratory scale under controlled conditions in a number of previous studies such as Malley (1995), Henderson *et al.* (2010), Karhu *et al.* (2014), Yap *et al.* (2014) and Rao *et al.* (2018a, 2021). Specifically, Henderson *et al.* (2010) evaluated the ability of PosiDAF to separate *Microcystis aeruginosa* cells at different initial cell concentrations from water using poly(*N,N*-diallyl-*N,N*-dimethylammonium chloride) (PDADMAC) with the successful separation of >95% despite variability in the initial algal concentration, which was comparable to or better than conventional DAF. Subsequently, polymers derived from poly(*N,N*-dimethylaminoethyl methacrylate) (PDMAEMA) and PDADMAC were hydrophobically modified to improve polymer–bubble–cell interactions and reduce polymer residuals in the effluent (Yap *et al.* 2014; Rao *et al.* 2018a), with the aim of further decreasing the chemical dosing and residual chemical separation costs as well as the environmental footprint typically associated with the conventional coagulation–flocculation and DAF processes (Vandamme *et al.* 2013; Coons *et al.* 2014). These derivatives were subsequently tested on certain algal and cyanobacterial species exhibiting comparable separation effectiveness to commercially available PDADMAC (Yap *et al.* 2014; Rao *et al.* 2018a). Thus, the success of PosiDAF for algae and cyanobacteria separation was demonstrated at bench-scale.

The investigation of the PosiDAF process has been limited to bench-scale jar tests using synthetic water samples. Though the results of jar testing for modified-bubble DAF can yield insights into the process performance and operational requirements, there are fundamental differences between a DAF jar tester and a continuous flow DAF plant. As an example, Lundh *et al.* (2002) investigated the effect of contact zone construction on flow in a DAF reactor at pilot scale. It was found that the removal of contaminants can be impacted greatly depending on the depth and shape of the contact zone; variables that cannot be changed in jar tests. In addition to this, the DAF recycle flow is diverted from the clarified water; thus, any contaminants that

carry over from DAF are recycled back into the process via the saturator. Furthermore, if a pilot plant is installed on the site of the source water, the plant is subjected to the natural variable water quality and any processes that are applied can be more easily translated to the full-scale operation. Hence, the investigation of the PosiDAF process at pilot-scale is critical to assess the feasibility of the process outside of laboratory conditions.

Until now, with the exception of preliminary summary data published by the authors (Henderson *et al.* 2015), no thorough evaluation of PosiDAF at pilot-scale has been published, nor has the process been tested using real, environmental samples rather than laboratory-grown monocultures. Hence, the aim of this study was to investigate the translation of the previous innovations to real large scale systems and so understand the potential for bubble modification as a means for practical algal separation.

2. MATERIALS AND METHODS

2.1. Chemicals

Unless otherwise stated, all chemicals were obtained from Sigma-Aldrich and used without any further purification. Low M_w (100,000–200,000 g/mol) PDADMAC (20 wt% in H₂O) was lyophilised prior to use. 2-(*N,N*-dimethylamino)ethyl methacrylate (DMAEMA, Aldrich, 98%) was passed through a basic alumina column to remove inhibitor prior to use. 2,2'-Azobisisobutyronitrile (AIBN, Wako Chemicals, 98%) was re-crystallised twice from methanol.

2.2. Synthesis of the hydrophobically modified PDMAEMA polymer (HMP)

Following the same procedure as in previous studies (Yap *et al.* 2014; Rao *et al.* 2018a), PDMAEMA and the best performing quaternised PDMAEMA derivative from those studies were synthesised. Briefly, the homopolymer was synthesised by simple bulk free radical polymerisation using AIBN as the initiator. Following the synthesis of the homopolymer, its quaternisation was carried out with 1-bromodecane at room temperature to obtain the HMP. Both these polymers were lyophilised prior to use.

2.3. Test site

The Bolivar Wastewater Treatment Plant Adelaide, South Australia (SA), Australia, treats municipal wastewater using activated sludge reactors, followed by WSPs that have a residence time of 14 days. The WSP effluent then feeds into a dissolved air flotation/filtration (DAF/F) plant as a final treatment stage before it is ready for recycling applications. The WSP effluent was therefore used as the water matrix for both bench- and pilot-scale conventional DAF and PosiDAF experiments. Refer to Supplementary Material, section S1.1 for more details on the WSP effluent background matrix characteristics during the testing period.

2.4. Bench-scale PosiDAF jar testing

2.4.1. Water samples for testing

Two strains of *M. aeruginosa*, CS-555/1 and CS-564/01, were obtained from the Commonwealth Scientific and Industrial Research Organisation's (CSIRO) Australian National Algae Culture Collection (ANACC) facility (Hobart, Australia). The species were re-cultured and grown in MLA media according to the protocols established previously (Rao *et al.* 2018b).

Both strains were harvested at the late exponential phase of growth (12–14 days after culturing) to provide an algal-rich sample for testing. The cells were diluted to 7.5×10^5 cells mL⁻¹ with the 100 μm filtered WSP effluent background matrix, and the pH was corrected to 7 using 1 M HCl and 1 M NaOH.

2.4.2. PosiDAF jar test procedure

Bench-scale PosiDAF jar tests were conducted to provide an indication of the dosing ranges for the pilot-scale experiments. Jar tests were conducted using a DBT6 (EC Engineering, Canada) DAF Batch Tester following previously established procedures (Yap *et al.* 2014; Rao *et al.* 2018a). Both PDADMAC and the HMP were used as bubble-modification chemicals. The jar tests were conducted in batches of 1 L on the influent water from section 2.4.1. The saturator feed water was obtained from the effluent of the St Kilda DAF/F plant. Aliquots of saturator feed water were dosed with either PDADMAC or the HMP as appropriate, up to a concentration of 50×10^{-3} meq L⁻¹ (or 15 mg L⁻¹), prior to the pressurisation at 450 kPa. A recycle ratio of 20% was used for all tests and effluent samples of 50 mL were collected after a 10-min flotation period. All solutions were adjusted to pH 7 using 1 M HCl and 1 M NaOH prior to jar testing. Assays were performed in triplicate using unique samples.

2.5. Single-train pilot-scale conventional DAF and PosiDAF jar testing

2.5.1. Conventional DAF testing procedure

Conventional operation of the single-train DAF pilot plant was carried out as per Yap *et al.* (2012) for comparison purposes between the pilot-scale and full-scale DAF processes. Briefly, the pilot plant was fed with the WSP effluent at a rate of $5 \text{ m}^3 \text{ h}^{-1}$. Aluminium sulphate (Orica, Australia) and cationic polymer Magnafloc™ LT510 (Solenis, Australia) were obtained from the St Kilda DAF/F plant and used for chemical pre-treatment at the pilot plant. The aluminium sulphate and Magnafloc™ LT510 were dosed into the influent before static mixing and flocculation, respectively. Flotation was conducted with a 15% recycle ratio to match the protocols at the full-scale plant at the time of operation. The DAF effluent was filtered via depth filtration with a sand filter and collected in a balance tank to feed the saturator. Assays were performed in triplicate using unique samples.

2.5.2. PosiDAF testing procedure

The single-train DAF pilot plant described by Yap *et al.* (2012) was reconfigured such that modified bubbles were generated by dosing polymer into the saturator to operate in the PosiDAF mode. The pilot plant was fed with the WSP effluent at a rate of $5 \text{ m}^3 \text{ h}^{-1}$ and the pH was not corrected as the difference in polymer performance was found to be marginal (Supplementary Material, Figure S1). The influent was diverted to feed directly into the flotation tank, bypassing the flocculator. A separation baffle was inserted between the flocculation and flotation tanks to prevent the flow of water between the tanks (Figure 1). A recycle ratio of 20% was used for flotation. The pilot plant was operated for 7 h each day over a period of 14 consecutive days. Each chemical was trialed over the course of 1 day and then changed the following day with all experiments undertaken within 14 days.

PDADMAC and the HMP were used for the pilot-scale PosiDAF testing and the dose ranges were selected based on the outcomes from the bench-scale PosiDAF study (section 2.3.3). Polymer addition was conducted with a DDI 209 digital dosing pump (Grundfos Alldos, Australia) after the recycle feed pump but upstream of the saturator. Polymer dosing ranges were determined by jar tests described in section 2.3.2.3. As charge demand has been shown to be linked to the coagulant dose required (Bernhardt & Schell 1993; Yap *et al.* 2014; Henderson *et al.* 2015), the charge demand was used as a metric to define the polymer dose.

PosiDAF effluent water was filtered via depth filtration with a sand filter and collected in a balance tank to feed the saturator. After the adjustment to polymer dosing set point, the plant was operated for 30 min to ensure the steady state. Two types of samples were collected for the effluent analysis: (1) grab samples of DAF influent and effluent water were then taken at 3-min intervals over the course of 15 min in 375 mL sample bottles, creating influent and effluent water sample sets. (2) A composite sample for further analysis was then created from

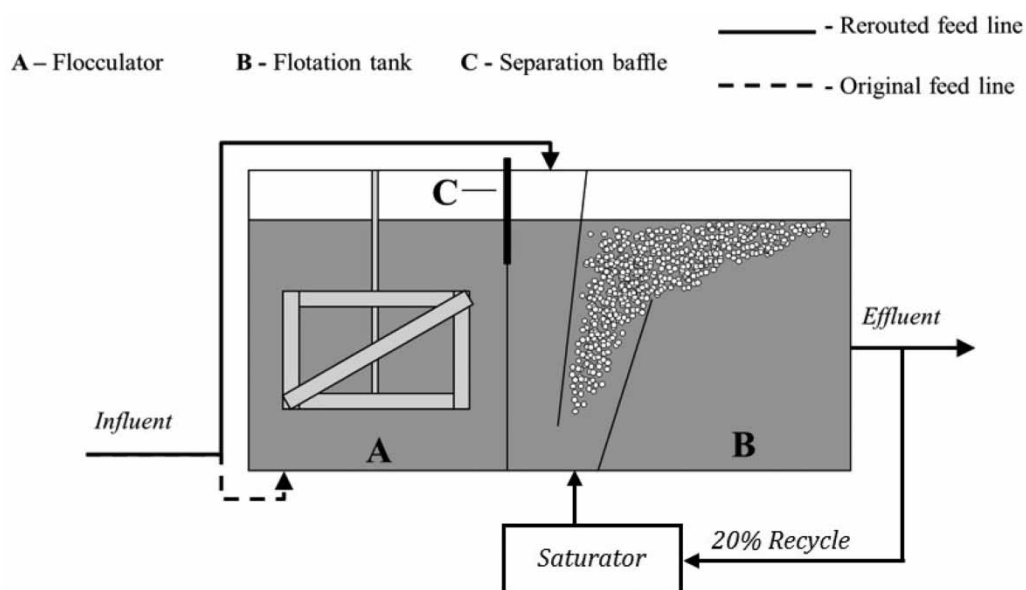


Figure 1 | Depiction of the modification to the flocculator–DAF unit in the pilot-scale DAF plant. ‘C’ denotes the location of separation baffle, installed for modified-bubble experiments.

each sample set by combining 20 mL from each of the five grab samples such that combined effluent water and combined influent samples were obtained. All assays were conducted in triplicate using unique samples.

2.6. Characterisation and analysis

2.6.1. Bench-scale jar testing

Influent: The WSP effluent matrix was subjected to analysis including: (a) the dissolved organic carbon (DOC) analysis with a Shimadzu TOC-Vsch total organic carbon (TOC) analyser (Shimadzu, Japan), (b) the zeta potential analysis with a Zetasizer Nano ZS (Malvern, Australia), and (c) the charge demand analysis with a Müttek PCD-04 laboratory charge demand analyser (BTG, Australia). The lab-grown cyanobacterial cells were characterised by size using a Mastersizer 3000 (Malvern, Australia) and zeta potential using a Zetasizer Nano ZS (Malvern, Australia).

Effluent: Analysis of the post-jar testing included cell counting using a haemocytometer or Sedgwick Rafter counting chamber and a DM500 light microscope (Leica Microsystems Ltd, Australia), and the zeta potential analysis using a Zetasizer Nano ZS (Malvern, Australia).

2.6.2. Pilot-scale jar testing

Influent analysis for conventional DAF and PosiDAF: WSP effluent (which is the St Kilda DAF/F influent) turbidity data at the St Kilda DAF/F plant were recorded via a Supervisory Control and Data Acquisition (SCADA) system for the DAF influent and unfiltered treated water. Measurements were made with continuous turbidimeters located on the plant influent line and sample line located above the filtration bed on a DAF/F unit. Algae and cyanobacteria concentrations and speciation data were obtained from SA Water, where all influent samples were also analysed for DOC using a Model 820 TOC analyser (Sievers Instruments Inc., USA).

Effluent: All conventional DAF and PosiDAF effluent water samples were subjected to the turbidity analysis, with a 2100N Laboratory Turbidimeter (Hach, Australia), and the turbidity measurements of the conventional DAF effluent were compared with the St Kilda DAF/F plant, acquired from the SCADA system. Cell counting in all samples was conducted with a haemocytometer and CH Series light microscope (Olympus, Tokyo, Japan). The composite effluent samples from pilot-scale PosiDAF were subjected to the charge demand analysis with a Müttek PCD-04 laboratory charge demand analyser (BTG, Australia).

For the off-site analysis, all conventional and PosiDAF effluent samples were filtered with a 0.45 µm polyethersulphone (PES) syringe filter (Millipore, Australia) and were analysed by the Australian Water Quality Centre (AWQC), SA Water, Adelaide, for UV-visible 254 nm measurements with a UV-vis spectrophotometer, Model 918 (GBC, Australia) and the DOC analysis with a TOC analyser, Model 820 (Sievers Instruments Inc., USA).

3. RESULTS AND DISCUSSION

3.1. Bench-scale experiments

3.1.1. Characterisation of the influent WSP effluent

The DOC of the WSP effluent background matrix was found to be $18 \pm 1 \text{ mg L}^{-1}$ (Table 1). With the addition of either *M. aeruginosa* CS-555/01 or CS-564/01 cells to WSP matrix, no change in DOC was observable (Table 1). In comparison to the DOC of the WSP effluent measured in a previous study (Yap *et al.* 2012), the DOC concentrations in the current study were 23% higher (Table 1). This could be due to the presence of particles in the water, which tends to release DOC over time as noted in several prior studies (Burdige & Komada 2015; Michael-Kordatou *et al.* 2015; Lu *et al.* 2020).

The WSP effluent background matrix was found to carry a negative charge with a zeta potential of $-6.5 \pm 0.9 \text{ mV}$ and a charge density of $-25 \pm 1 \times 10^{-3} \text{ meq L}^{-1}$ (Table 1). Upon combination with either CS-555/01 or CS-564/01, the zeta potential became more negative (Table 1); however, the charge demand of both the

Table 1 | Characterisation of influent feedwater for DAF jar tests

Water type	Zeta potential (mV)	Charge demand ($\mu\text{eq L}^{-1}$)	DOC (mg L^{-1})
WSP background matrix	-6.5 ± 0.9	-25 ± 1	18.1 ± 0.5
WSP matrix + CS-564/01 cells	-21 ± 2	-25 ± 1	18.2 ± 0.5
WSP matrix + CS-555/01 cells	-11 ± 1	-22 ± 1	17.8 ± 0.4

species remained comparable upon the addition of the respective cells (Table 1). This was interesting as while the dilution of the WSP matrix was less than 5% upon the addition of cells, it resulted in a comparable charge demand, despite the zeta potential becoming more negative.

3.1.2. Bench-scale PosiDAF

Maximum cell separation of the *M. aeruginosa* strain CS-564/01 in the WSP effluent using the PosiDAF bench-scale jar testing was found to be $95 \pm 2\%$ and $92 \pm 1\%$ using PDADMAC and the HMP, respectively (Figure 2). The cell separation of the equivalent for CS-555/01 cells was less effective, reaching a maximum cell separation of only $41 \pm 3\%$ and $48 \pm 2\%$ for PDADMAC and the HMP, respectively (Figure 2). It is notable that the cell separation of CS-555/01 was greater in the presence of WSP matrix compared to the buffer solution in prior work, where a maximum cell separation of only 31% was obtained (Yap *et al.* 2014; Rao *et al.* 2018a, 2018b). In addition to differences in cell separations, the doses required to achieve maximum cell separations were found to be much greater when the filtered WSP effluent was used as a background matrix as opposed to that obtained in the previous research when using a buffer as background matrix (Yap *et al.* 2014; Rao *et al.* 2018a, 2018b). When using PDADMAC with CS-564/01 cells, cell separations above 95% were achieved at a dose of 17×10^{-3} meq L⁻¹ (Figure 2), compared to tests using a matrix of only buffered Milli-Q water, where a dose of only 0.7×10^{-3} meq L⁻¹ was required (Yap *et al.* 2014; Rao *et al.* 2018a, 2021), demonstrating that the WSP background matrix significantly increased the polymer demand. Overall, analysis of results from these tests demonstrated that the recommended doses for the pilot-scale study were a maximum of 8.0 mg L⁻¹ (0.041 meq L⁻¹) for PDADMAC and 10 mg L⁻¹ (0.034 meq L⁻¹) for the HMP.

Applying PosiDAF with the HMP resulted in a more negative DAF effluent residual charge than with PDADMAC for the same charge dose (Figure 2). To illustrate, the resulting equivalent zeta potentials, for a 20×10^{-3} meq L⁻¹ dose of PDADMAC and the HMP, were found to be -12.5 and -17.8 mV for CS-564/01, respectively, and -10.8 and -15.2 mV for CS-555/01, respectively (Figure 2). In prior work using a buffered Milli-Q water as the background matrix, this difference in zeta potential was found to be much more significant, with differences of up to 45 mV (Yap *et al.* 2014; Rao *et al.* 2018a). In contrast to previous results and despite doses up to 50×10^{-3} meq L⁻¹ of PDADMAC, DAF effluent water remained negatively charged (Figure 2), possibly due to a lack of residual polymer in the effluent.

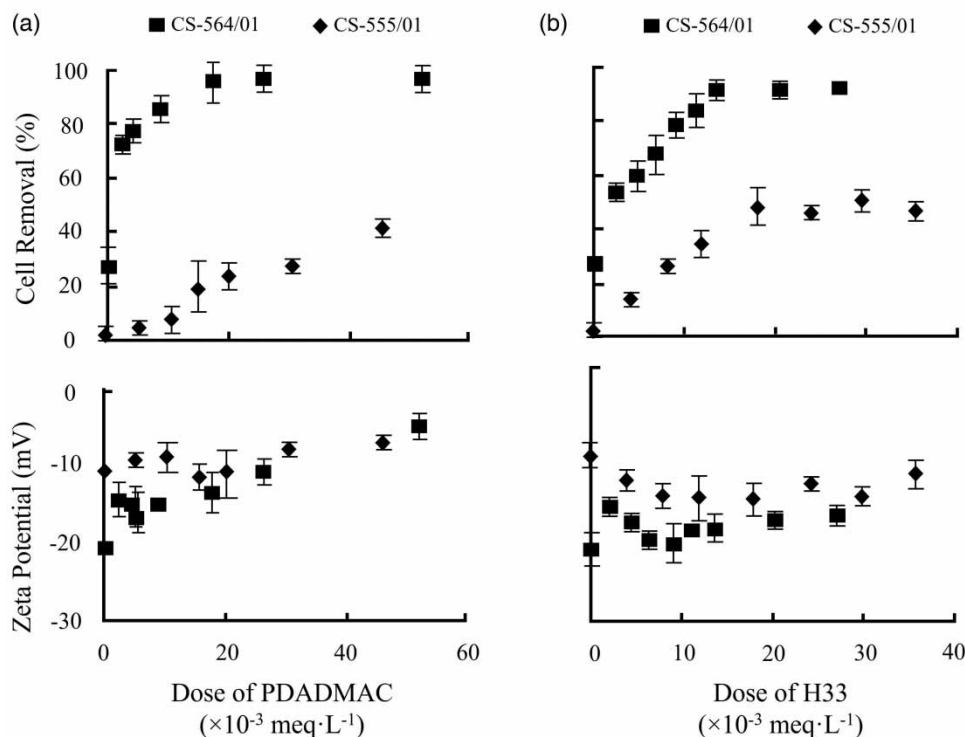


Figure 2 | PosiDAF experiments at bench-scale using PDADMAC and the HMP to separate *Microcystis aeruginosa* CS-564/01 and CS-555/1 cells dispersed in a background matrix of the WSP effluent obtained from the Bolivar Wastewater Treatment Plant.

The decrease in WSP matrix charge when combined with CS-555/01 (Table 1), followed by improved cell separation compared to previous results using buffered Milli-Q (Yap *et al.* 2014; Rao *et al.* 2018a) suggests that the dissolved organic matter in the matrix complexes with the cells, complimenting cell–bubble attachment. Previously, PosiDAF performance was enhanced with the addition of specific algal organic matter (AOM) (Rao *et al.* 2018b), indicating that the AOM presents aids in cell separation using PosiDAF. Successful cell separation under highly anionic conditions indicates the predominance of non-Derjaguin, Landau, Vervey, and Overbeek (DLVO) forces such as bridging interactions between cells and AOM. This has also been observed during coagulation and flocculation for particle separation in which the AOM was suggested to contribute to these forces (Sharp *et al.* 2005; Pivokonsky *et al.* 2015, 2016; Baresova *et al.* 2017).

3.2. Pilot-scale experiments

3.2.1. Characterisation of the conventional DAF and PosiDAF influent

As the influents for the conventional and PosiDAF were the same, the influent water quality data between the processes were comparable (Table 2). The average turbidity of the conventional DAF and PosiDAF influents to the pilot plant (i.e., the WSP effluent) was skewed by occasional turbidity spikes, leading to the saturation of the sensor and of the records on the SCADA system (Supplementary Material, Figure S3). Using 2 h sample points, it was found that the median influent water turbidity was 90 ± 412 NTU (conventional DAF) and 127 ± 373 NTU (PosiDAF) during the pilot-scale trial in the current study, which was much greater than that observed in a previous pilot-DAF study in which an average of 41 NTU was observed (Table 2, Supplementary Material, Figure S2) (Yap *et al.* 2012). Over the duration of the pilot plant trials, algal population densities remained high with total cell concentrations ranging from 4.6×10^5 to 5.2×10^6 cells mL⁻¹ and concentrations of *Microcystis flos aquae* contributing between 46 and 97% of total cells (Table 2, Supplementary Material, Figure S2). Other dominant species over this period include *Stichococcus*, *Planktolyngbya*, *Aphanocapsa*, *Chlorella*, *Dimorhococcus*, and *Planctonema* (Supplementary Material, Table S1). As the predominant cause of turbidity was low density algae and cyanobacteria, the relationship between the two was found to be roughly linear, and hence, the turbidity can be used as a surrogate for the cell concentration in this study (Supplementary Material, S3).

3.2.2. Conventional DAF at pilot-scale

The pilot plant was operated initially using conventional DAF conditions, including coagulation and flocculation to compare pilot plant performance with the St Kilda DAF/F plant. It was found that the reduction in the effluent turbidity at the pilot plant was greater than that of the full-scale plant prior to filtration, with average DAF effluent water turbidities of 1.7 ± 1.2 and 6.1 ± 1.8 NTU, respectively (Supplementary Material, S4). In both plants, similar fluctuations in DAF effluent water quality were observed, with fluctuating DOC removals in the range of 12–22%. The average turbidity and cell removal at the pilot plant were $97.9 \pm 1.3\%$ and $88.5 \pm 4.5\%$, respectively

Table 2 | Influent water quality indicators for full-scale DAF, pilot-scale DAF, and PosiDAF, and the aluminium sulphate dose for full-scale DAF measured at the St Kilda DAF/F plant, fed from the WSP system throughout the testing period

Property	Units	Experiment duration (February 2013)		
		Full-scale DAF	Pilot-DAF	Pilot PosiDAF
Turbidity	NTU	86 ± 384	90 ± 412	121 ± 391
Charge demand	meq L ⁻¹	-29 ± 3	-26 ± 5	-31 ± 4
Total cell count ^a	cells mL ⁻¹	$4.68 - 4.79 \times 10^6$	$4.11 - 4.69 \times 10^6$	$4.60 - 5.23 \times 10^6$
Dominant species (>45% population)		<i>Microcystis flos aquae</i>		
pH		8.6 ± 1.3	8.1 ± 0.6	8.8 ± 1.4
Temperature	°C	21.5 ± 3.4	22.3 ± 1.6	21.9 ± 4.1
DOC	mg L ⁻¹	13.6 ± 0.3	14.6 ± 0.7	13.1 ± 0.7
Specific UV absorbance	m ⁻¹ mg ⁻¹ L	2.18 ± 0.09	2.12 ± 0.28	2.33 ± 0.13
Aluminium sulphate dose ^b	mg L ⁻¹	13.1 ± 5.6	–	–

^aRecords from analysis of weekly samples at AQWC, SA water.

^bDose quoted as Al.

(Table 2, Supplementary Material, Figure S4). However, the pilot plant was more susceptible to sudden spikes of turbidity in the influent water in comparison to the St Kilda DAF/F plant, resulting in an increase of the DAF effluent water turbidity. For example, in one incident, elevated turbidity and cell concentrations led to rapid filter fouling and turbidimeter measurement saturation at the pilot plant, but not at the full-scale plant, despite using the same coagulant dose (Supplementary Material, Figure S4). Similar fluctuations in cell removals have been observed in other DAF pilot plants that have utilised coagulants, such as alum and ferric chloride (Marston & VandeVenter 2015), suggesting that the fluctuations could be dependent on the influent rather than the coagulant used. The stability of the effluent turbidity at the St Kilda DAF/F plant (prior to filtration) is likely attributed to its buffering capacity, whereby sufficient particle coagulation could be maintained in the flocculators given a longer hydraulic retention time (Marston & VandeVenter 2015; Besson *et al.* 2019).

3.2.3. PosiDAF at pilot-scale

3.2.3.1. Cell and turbidity separation. Analysis of pilot-scale PosiDAF experiments showed that >95% cell separation was obtained at a dose of 12×10^{-3} meq L⁻¹ using PDADMAC (Figure 3(a)). Over the course of the experimental run (7 h per day for 14 days), an average cell separation of $98 \pm 1\%$ was achieved for all doses greater than 12×10^{-3} meq L⁻¹, with a maximum cell separation of $99 \pm 1\%$ (Figure 3(a)). In comparison, bench-scale PosiDAF jar tests resulted in similarly high cell separations, reaching a maximum of 96.3%, but required 56% more polymer to achieve this cell separation (Figure 2). The optimal turbidity reduction in pilot-scale PosiDAF with PDADMAC occurred at doses of 19.5×10^{-3} meq L⁻¹ and greater, averaging $95 \pm 2\%$ and reaching a maximum of $98 \pm 2\%$ (Figure 3). This was found to be comparable to the

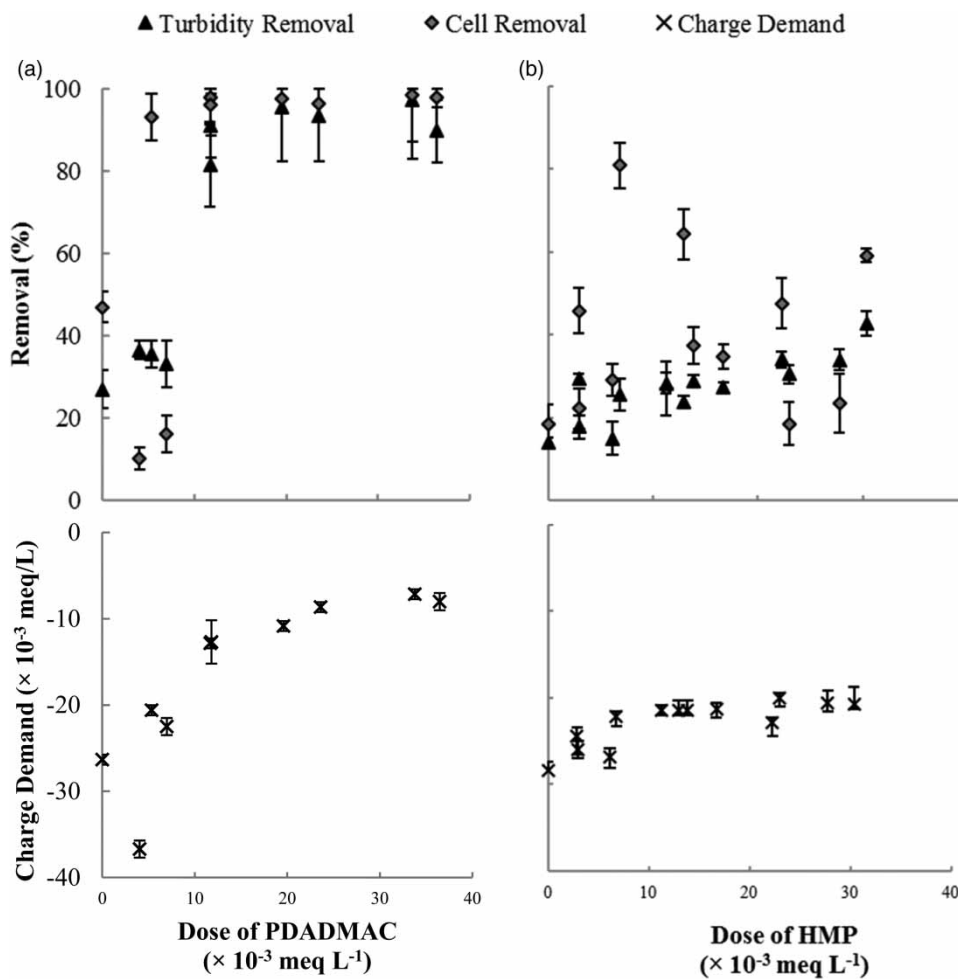


Figure 3 | Removal of cells and turbidity using pilot-scale PosiDAF with (a) PDADMAC and (b) HMP.

pilot-scale conventional DAF performance where the average cell separation over 4 days and 142 data points was $98 \pm 1\%$ (Figures 2 and 3, Supplementary Material, Figure S4) (Yap *et al.* 2012).

The use of HMP in pilot-scale PosiDAF experiments resulted in much poorer performance compared to PDADMAC (Figure 3). A maximum turbidity reduction of $43 \pm 3\%$ was achieved at a dose of $30 \times 10^{-3} \text{ meq L}^{-1}$ (Figure 3(b)). This contrasted with bench-scale laboratory results where both the HMP and PDADMAC produced comparable results (Figure 2). Cell separations in this trial were generally $<50\%$, although they reached as high as $81 \pm 6\%$ at a dose of $6.8 \times 10^{-3} \text{ meq L}^{-1}$ (Figure 3(b)).

3.2.3.2. DOC separation. Unlike turbidity, which was stable in the pilot-scale PosiDAF tests, DOC separation did not remain steady (Figure 4). For the dosing range tested, a maximum of $23.9 \pm 0.5\%$ DOC separation was obtained using $33 \times 10^{-3} \text{ meq L}^{-1}$ PDADMAC while when using the HMP, a maximum of $8.8 \pm 0.9\%$ DOC separation was obtained (Figure 4). This was slightly lower than that obtained during the conventional DAF operation (Yap *et al.* 2012). Over the course of the pilot plant operation, DAF influent SUVA remained low, ranging between 2.13 and $2.58 \text{ m}^{-1} \text{ mg}^{-1} \text{ L}$ (Table 2), indicating that the concentration of readily treatable DOC was low in regard to separation processes using metal coagulants (Edwards 1997; Duan & Gregory 2003; Edzwald 2010; Matilainen *et al.* 2010). This was demonstrated in comparison tests between conventional DAF and PosiDAF in which a maximum DOC separation of only 22% was achieved for the PosiDAF tests (Figure 4). For conventional water treatment with coagulation–flocculation, it is known that highly cationic polymers are more effective for DOC separation than cationic polymers with lower charge (Bolto *et al.* 1999; Bolto & Gregory 2007). This observation was mirrored in pilot-scale PosiDAF tests, with the highly cationic PDADMAC outperforming moderately charged HMP (Figure 4).

3.2.3.3. Effluent charge characteristics. The charge of the PosiDAF effluent water from the pilot plant was found to be negative for all polymer doses, irrespective of the polymer trialled, as it was in the jar testing (Figure 3). At doses $>10 \times 10^{-3} \text{ meq L}^{-1}$, the charge of the PosiDAF effluent water was more anionic for the HMP (Figure 3(b)) compared to PDADMAC (Figure 3(a)). For both polymers, a plateau in the charge was apparent beyond $23 \times 10^{-3} \text{ meq L}^{-1}$ (PDADMAC) and $11 \times 10^{-3} \text{ meq L}^{-1}$ (HMP). In experiments with the HMP, the most negative charge observed was $-20 \pm 2 \times 10^{-3} \text{ meq L}^{-1}$, obtained at a dose of $30 \times 10^{-3} \text{ meq L}^{-1}$ (Figure 3(b)), whereas most negative charge for PDADMAC was $-7 \pm 1 \times 10^{-3} \text{ meq L}^{-1}$ at a dose of $33 \times 10^{-3} \text{ meq L}^{-1}$ (Figure 3(a)). The charge observations in Figure 3 are comparable to those obtained in bench-scale PosiDAF

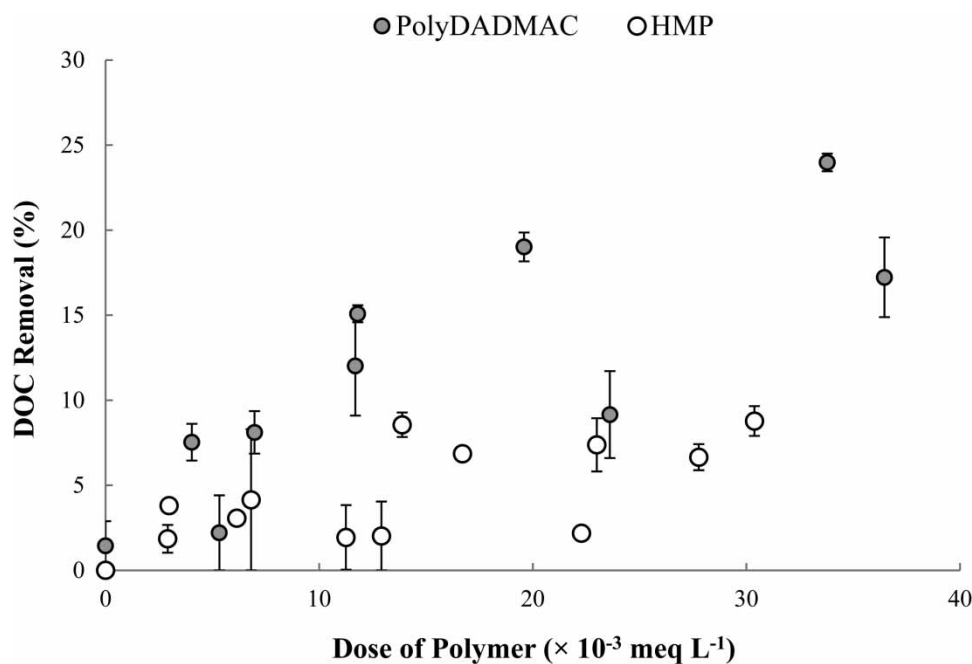


Figure 4 | Removal of DOC using pilot-scale PosiDAF with PDADMAC (grey circles) and the HMP (white circles).

jar tests conducted in this work and prior research (Yap *et al.* 2014; Rao *et al.* 2018a), which is indicative of an effective translation of previous laboratory trials to large scale pilot systems for treating algae-laden waters.

3.2.4. Comparison of float characteristics between pilot-scale conventional DAF and PosiDAF

An interesting feature of the pilot-scale PosiDAF is the nature of the float, which was very different to that observed when operating under pilot-scale conventional DAF (Figure 5). Images of the PosiDAF float at pilot-



Figure 5 | Images of the float layer produced using (a) PosiDAF with PDADMAC, (b) PosiDAF with the HMP, and (c) conventional DAF. In PosiDAF, the float is removed as a sheet, whereas in conventional DAF, the float was observed to break up due to the shear forces during hydraulic removal.

scale revealed that the float developed as a foamy layer irrespective of whether PDADMAC or the HMP was applied (Figure 5(a) and 5(b)). During hydraulic float removal, both the PDADMAC and HMP PosiDAF float layers behaved as a single agglomerated sheet with 6.5–7.9% solids (Figure 5(a) and 5(b)), as opposed to discrete agglomerated clusters observed with conventional DAF where the solid content was only 3.4–4.6% (Figure 5(c)). In the development of a float via the PosiDAF process, particles and polymer are concentrated on the top of the DAF tank whereby further inter-particle bridging can take place with the AOM, resulting in a single uniform float with a foamy consistency. These observations suggest the potential benefits of float generation using the PosiDAF process in comparison to the conventional DAF process.

Differences were also observed among the floats generated during the PosiDAF process. In the HMP PosiDAF with much lower cell separation, much greater bubble coalescence was observed, with much larger bubbles present in the float (~6.6% solids) (Figure 5(b)), compared to PDADMAC PosiDAF, which experienced minimal bubble coalescence, with the float layer devoid of large coalescing bubbles (~7.9% solids) (Figure 5(a)). These observations are in contrast with those made in a previous study where it was observed that PDADMAC adsorption onto the bubbles resulted in bubble coalescence when the PDADMAC concentration was $>10 \times 10^{-3} \text{ meq L}^{-1}$ (2 mg L^{-1}), whereas the HMP strongly adsorbed onto the microbubble surface with increasing polymer concentrations (Rao *et al.* 2018a). It is to be noted that the assays conducted in the previously published study were on bubble-polymer interactions in Milli-Q water, while the pilot study was conducted on environmental samples, which inherently have organic matter that could influence polymer adsorption on the bubble surface. Given that the HMP and not PDADMAC was impacted by the presence of the organic matter, and the effluent charge is negative for both the polymers, it is suggested that polymer properties such as self-assembly could prevent HMP-bubble interactions. Overall, the coalescence of HMP-coated bubbles indicated that these bubbles were less stable than the PDADMAC-coated bubbles, and hence, could be a reason behind the muted performance of the HMP in pilot PosiDAF.

3.3. Translating PosiDAF from bench- to pilot-scale: important considerations

Upscaling PosiDAF from bench- to pilot-scale was generally successful. PDADMAC resulted in similar ($t < 1$) cell separations at all polymer concentrations irrespective of whether it was applied in bench- or pilot-scale, thereby demonstrating that the PosiDAF application on a large scale was feasible (Figures 2 and 3). However, when using the HMP, there was a significant difference ($t > 10$) in cell separation effectiveness reported between bench- and pilot-scale PosiDAF (Figures 2 and 3). As all PosiDAF process parameters and influent water quality were similar in both, bench- and pilot-scale work, the reasons for the discrepancy in process efficiencies could be due to: (a) differences in flotation tank dimensions between bench- and pilot-scale PosiDAF, (b) recycle water quality, and (c) polymer-bubble interactions. These will be systematically explored to identify the root cause.

3.3.1. Influence of flotation tank dimensions on flotation efficiency

An important difference between the bench- and pilot-scale PosiDAF processes was in the dimensions of the flotation jar (bench-scale) and tank (pilot-scale); height to width ratio of the jar was 3.5, whereas the same for the tank was 1.9. This would imply that the wider pilot-scale tank had a bigger turbulent mixing zone and a smaller plug-flow zone in the axial direction (depth) compared to the narrow jar (Lundh *et al.* 2002; Edzwald 2010; Fanaie *et al.* 2019). This would result in differing bubble-particle contact zone configurations in bench- and pilot-scales, thereby, causing discrepancies in efficiencies (Fanaie *et al.* 2019). However, it was seen that only the HMP showed fluctuating outcomes between bench- and pilot-scales, whereas PDADMAC had >95% efficiency in both experiments. Hence, this suggests that the influence of varying contact zone configurations on flotation efficiencies was not apparent in this study.

3.3.2. Influence of recycle water quality on pilot-scale PosiDAF efficiencies

Another difference between the bench- and pilot-scale processes was the quality of the recycle water. The saturator water used in the bench-scale jar tester had already been treated by C-F, DAF, and filtration, and hence, the likely presence of any DOC in the saturator water would be minimal (Edwards 1997; Duan & Gregory 2003). Consequently, the bench-scale PosiDAF application using both polymers resulted in >90% separation efficiencies (Figure 2).

In comparison, the saturator water in the pilot plant was not treated by C-F, DAF, and filtration, and hence, contained residual DOC that was not effectively removed during the pilot PosiDAF process, as observed in Figure 4. This DOC would be available to complex with the polymers upon polymer addition to the saturator,

thereby resulting in less polymer available for interactions with the bubbles and cells. While this could occur, it was evident from Figure 3 that the cell separation using PDADMAC remained unaffected while the HMP resulted in low cell separation, and that the effluent charge demand was significantly more negative for the HMP compared to PDADMAC. This suggests additional factors beyond the tank dimensions and recycle water quality that influence the pilot-scale PosiDAF cell separation.

3.3.3. Impact of polymer–bubble interactions on the pilot-scale PosiDAF efficiency

Significant bubble coalescence was observed in the HMP-treated float compared to the PDADMAC-treated float (section 3.3.4 and Figure 5), implying that HMP-coated bubbles were less stable than PDADMAC-coated bubbles. Possible reasons for the low stability of the HMP-coated bubbles are (a) low HMP adsorption onto the bubbles or (b) micellisation of the HMP on the bubbles causing low polymer coverage on the bubble surface. From previous studies (Yap *et al.* 2014; Rao *et al.* 2018a), it has been established that a negative effluent charge demand similar to that observed for both polymers in Figure 3 is indicative of strong polymer association with the bubbles. This suggests that the HMP micellisation on the bubble surface, which prevented it from interacting with the cells (due to high hydrophobicity), was the likely reason for the poor stability of the HMP-coated bubbles, and consequently, low flotation efficiency on pilot-scale. As for PDADMAC, during separation, the polymer adsorbed on the bubble surface, and bridged between cells, AOM, and the bubbles, resulting in effective separation in both bench- and pilot-scales (Figures 2 and 3).

3.4. PosiDAF process benefits

As described earlier, chemicals costs when applying coagulation–flocculation followed by DAF can fluctuate between 30 and 70% of the total process costs (Vandamme *et al.* 2013; Coons *et al.* 2014; Allnut & Kessler 2015; Dunlap *et al.* 2015; Demirbas 2017). In PosiDAF, chemical consumption was found to be significantly less compared to conventional DAF/F at Bolivar; conventional DAF uses both alum and PDADMAC (known as Magnafloc™ LT510), at a dosing range of 110–230 mg L⁻¹ (average dose: 161 mg L⁻¹) and 0.4–1.2 mg L⁻¹ (average dose: 0.6 mg L⁻¹), respectively, whereas PosiDAF uses only PDADMAC at a dosing range of 3–8 mg L⁻¹ (Table 3) (Coons *et al.* 2014; Henderson *et al.* 2015). At these dosing ranges and a cost of A\$240 tonne⁻¹ for alum and A\$1,800 tonne⁻¹ for PDADMAC (Henderson *et al.* 2015), Henderson *et al.* (2015) previously calculated the chemical costs to be 5.05–10.55 cents kL⁻¹ for alum and 0.27–0.80 cents kL⁻¹ for PDADMAC in conventional DAF, whereas 2.00–5.33 cents kL⁻¹ in PosiDAF. This results in a potential cost-saving of up to 74% in the PosiDAF process.

In addition to chemical-savings, process products from the WSP effluent treatment will have a different character due to the high (up to 8%) solid contents compared to conventional DAF which has an average of ~4.1% solids, although pumping needs must be considered. With the absence of metal coagulants and much lower masses of treatment chemical used, the waste stream produced in PosiDAF is richer in organic materials. This has the potential to improve the biomass quality and sludge digestion owing to the reduction of alum content in the sludge (Sukias & Craggs 2011; Henderson *et al.* 2015). Additionally, polymer interactions with the cells during an anaerobic biomass digestion can enhance the generation of methane; it is to be noted that polymer concentrations of >15 g kg⁻¹ as dry solids in the biomass can inhibit the digestion performance (Chu *et al.* 2003). With dissolved P concentrations dependent on coagulating metal salts (Arnaldos & Pagilla 2010), dissolved P would remain in product water, which can be of benefit for agricultural and horticultural use

Table 3 | Chemical cost (cents kL⁻¹) and consumption (mg L⁻¹) at the Bolivar DAFF plant and modified-bubble flotation pilot plant fed with the WSP effluent over the course of the trial period

Chemical Unit	Conventional treatment						Modified-bubble flotation	
	Alum		LT 510		Combination		LT 510	
	mg L ⁻¹ Used	Cents kL ⁻¹	mg L ⁻¹ Used	Cents kL ⁻¹	mg L ⁻¹ Used	Cents kL ⁻¹	mg L ⁻¹ Used	Cents kL ⁻¹
Lowest	110	5.05	0.4	0.27	110.4	4.27	3	2.00
Highest	230	10.55	1.2	0.80	231.2	9.16	8	5.33
Average	161	7.39	0.6	0.40	161.6	6.25	5.5	3.67

especially in countries such as Australia (Shilpi *et al.* 2018). Further work is required to fully elucidate the impact on sludge character, for example, dewaterability and pumping considerations.

For the full-scale plant, average doses were calculated from alum and polymer dose values every 10 min over this period as recorded on the SCADA system. For the pilot plant, average doses were determined based on experimental data.

4. CONCLUSION

The current study provided novel information concerning the scale up of an innovative DAF process that utilises the polymer modification of bubbles as opposed to precoagulation of the solids. The results demonstrate the effective translation of previous laboratory trials to large scale pilot systems treating site wastewater including its inherent variability. An average cell separation of 98% was observed for PosiDAF compared to 88.5% with the conventional set up. In addition, new insights were provided relative to the float characteristic, which was observed to be structurally coherent and at a higher solid fractions of 6.6–7.9% for PosiDAF compared to ~4% in the conventional set up.

Economic translation of the findings suggests a conservative cost-reduction of 74% based on chemical-saving alone with additional benefits in relation to the sludge transport and processing. Key new insights were revealed about the importance of the AOM–polymer interactions in delivering resilient/effective separation and the role the polymer design has within this. Overall, the findings provide the clear illustration of the potential of PosiDAF, but further work is required on the role of AOM and the characterisation of the sludge float to deliver confidence for further implementation.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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