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Algal bioproducts derived from suspended solids in intensive land-based aquaculture

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HIGHLIGHTS

- ▶ We investigate the properties of suspended solids produced in land-based aquaculture systems.
- ▶ Harvested suspended solids were rich in valuable ω-3 and ω-6 fatty acids.
- ▶ Suspended solids offer potential as a bioresource for the production of biochar.
- ▶ Utilising suspended solids has potential to provide economic and environmental benefits.

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ABSTRACT

Land-based aquaculture produces suspended solids in culture pond and settlement pond waters that could be harvested as a bioresource. Suspended solids were quantified, characterised and harvested from these two sources to assess their suitability for conversion to bioproducts. The suspended solids of settlement ponds were less concentrated (87.6 ± 24.7 mg L^{-1}) than those of culture ponds (131.8 ± 8.8 mg L^{-1}), but had a higher concentration of microalgae ($27.5 \pm 4.0\%$) and consequently higher particulate organic carbon ($24.8 \pm 4.7\%$) and particulate nitrogen ($4.0 \pm 0.8\%$). The microalgal community also differed between sources with a higher concentration of fatty acids in the biomass from settlement ponds. Consequently, biochar produced from biomass harvested from settlement ponds was higher in organic carbon and nitrogen, with a lower cation exchange capacity. In conclusion, we characterised a renewable and potentially valuable bioresource for algal bioproducts derived from suspended solids in intensive land-based aquaculture.

1. Introduction

Intensive land-based aquaculture farms primarily operate under 'bioflocculation' regimes which comprise of an organic-rich biofloc composed of a suspended solid biomass of algae, bacteria or zooplankton (Burford et al., 2004). This biofloc provides the benefit of additional nutrition for culture species, above that of formulated feed inputs and progressive improvements in biofloc technology have enabled improved nutrition and improved discharge water quality (Burford et al., 2004; Jones et al., 2001). However, suspended solids which are not consumed by the culture species are subsequently released as a discharge and are considered a waste product. Suspended solids in aquaculture are traditionally treated in settlement ponds prior to release to the environment. However, the settlement efficacy of solids within these ponds is low in saline systems, with up to 40% of solids remaining in suspension (Jackson et al., 2003; Jackson et al., 2004). In addition, these solids ultimately form a nutrient-rich sludge which releases dissolved nitrogen back to the water column through microbial decomposition (Preston et al., 2001; Burford and Lorenzen, 2004). An alternative to the release or internal recycling of suspended solids is their capture and re-use as bioproducts (Jones et al., 2001).

The effective capture (harvest) of suspended solids is dictated by the concentration and particle size distribution of the suspended solids in the discharge water (Cripps and Bergheim, 2000). Suspended solid concentration and particle size distribution have been well documented in temperate aquaculture, including freshwater salmon (Kelly et al., 1997; Cripps, 1995) and trout

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farms (Maillard et al., 2005), and are dilute in concentration (1.5–8.0 mg L^{-1}) and predominantly small in size (<30 μm) (reviewed by Cripps and Bergheim, 2000). In contrast, particle size distribution warrants further investigation in wastewater from tropical land-based aquaculture farms as operations intensify and wastewater treatment techniques advance.

Importantly, a large proportion of suspended solids in tropical land-based systems can be microalgae, a rich source of lipids and extractable fatty acids. Microalgal fatty acids have applications in aquaculture feeds and nutraceuticals (Spolaore et al., 2006), and as a biofuel through trans-esterification to biodiesel (Biller and Ross, 2011). The quality and quantity of fatty acids varies greatly between classes and phyla of microalgae (Huerlimann et al., 2010) and quantifying the yield and quality of microalgal biomass, including fatty acid composition, is a critical first step in evaluating the potential of suspended solids as a bioresource.

An alternative to the extraction and utilisation of fatty acids is the *in toto* utilisation of lipid rich (high calorific value) microalgal biomass through thermo-chemical conversion by pyrolysis. Slow pyrolysis of biomass produces energy and biochar, and is an effective waste mitigation tool (Panwar et al., 2012). Biochar production sequesters carbon and can mitigate CO_2, CH_4 and N_2O emissions on large scales (Read, 2008). The addition of biochar to soils improves soil structure and fertility, and enhances agricultural production (Steiner et al., 2007). Biochar produced from macroalgal biomass has high ash, nitrogen and extractable inorganic nutrient content, and excellent agricultural properties (Bird et al., 2011; Bird et al., 2012). Consequently, quantifying the yield and quality of biochar from microalgal biomass is an important additional assessment in evaluating the potential of suspended solids harvested from intensive land-based aquaculture as a bioresource.

Therefore, the first aim of this study is to quantify, characterise, and subsequently harvest the suspended solids from two discharge waste streams in pond-based intensive aquaculture. The first is the water from the culture ponds and the second the water from the settlement ponds. The second aim is to quantify the fatty acid profile of harvested biomass (suspended solids) and evaluate its potential as a bioproduct in aquaculture feeds and nutraceuticals. The third aim is to convert the harvested biomass (suspended solids) to biochar and evaluate its potential in soil amelioration. Finally, we discuss the scale of this bioresource and scope for its application.

2. Methods

2.1. Study site

All experiments were conducted at Pacific Reef Fisheries Ltd. in North Queensland, Australia (S19°28′46″, E147°29′18″). This farm has 73 ha of culture ponds with black tiger prawns (*Penaeus monodon*) situated in the dry tropics. At the time of sampling (May 2011), feeding rates were high with culture ponds at peak biomass production. Discharge water from the culture ponds was treated in traditional settlement ponds before being released to the environment. Water was collected from three operational culture ponds and the three operational settlement ponds (Fig. 1).

2.2. Initial assessment of suspended solid characteristics

Preliminary characterisation of suspended solids was conducted with particle size distribution and suspended solid concentration being quantified for three culture and settlement ponds on three

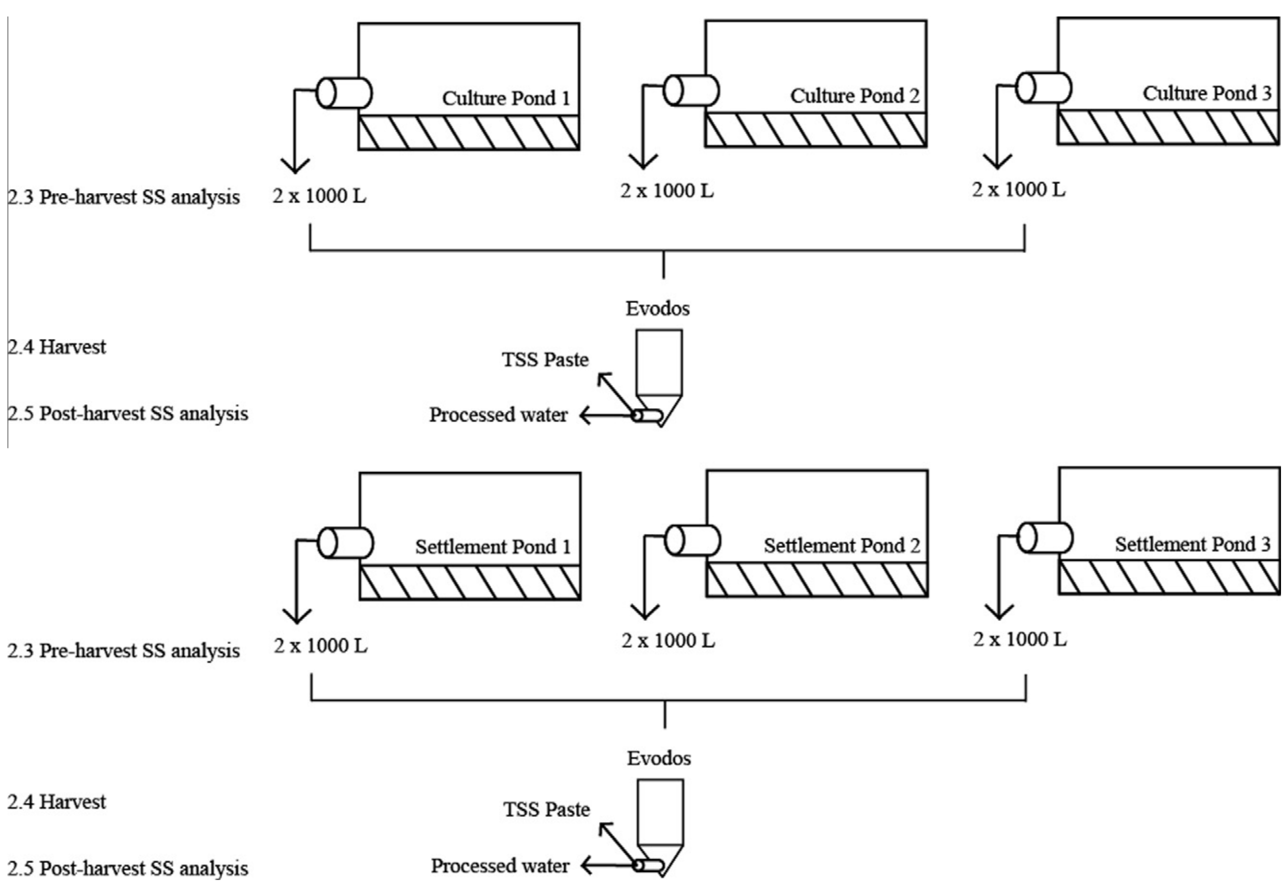

Fig. 1. A schematic of the protocol for analysing and harvesting suspended solid (SS) from the discharge of prawn farm culture and settlement ponds. The numbers denote the sub-headings in the methods section in the text.

days within one week (2–8th May 2011; see below for methods). Particle size distribution ranged between 0.03–400 μm across both pond types and the mean suspended solid load was 90.8 ± 8.2 mg L^{-1} in culture pond water and 65.3 ± 0.7 mg L^{-1} in settlement pond water. From this preliminary data it was estimated that 6000 L of water from each pond type was required to provide sufficient harvestable biomass for the characterisation of physico-chemical properties of the resulting algal pastes, the quantification of the fatty acid content, as well as the production and characterisation of biochar made from these pastes (Fig. 1).

2.3. Pre-harvest analysis of suspended solids

Water was collected from three operational culture ponds (3 × 2000 L) on 16th May 2011 and from three settlement ponds (3 × 2000 L) on 18th May 2011 (Fig. 1). The water from each pond was collected and transported in 2 × 1000 L intermediate bulk containers (IBC) (Fig. 1). Each IBC was used as a duplicate and water samples were collected from each IBC on filling for the characterisation of suspended solids. Suspended solid concentration, particulate nitrogen, particulate carbon, chlorophyll *a*, microalgae community composition and particle size distribution were quantified and characterised for each water sample.

Suspended solid concentration (mg L^{-1}) was quantified by filtering 250 mL of discharge water through a pre-weighed, 0.4 μm membrane filter and drying to a constant weight at 60 °C. To quantify particulate nitrogen and particulate carbon (as mg L^{-1} and converted to % of suspended solids) a known volume of water was filtered through pre-ashed (450 °C for 5 h) Whatman glass fibre filters (GFF), and stored frozen until analysis. Once thawed, filters were subjected to high temperature combustion in a Shimadzu TC-5000 fitted with a solid sample inlet. Chlorophyll *a* (determined as mg L^{-1} and converted to % of suspended solids) content was quantified by filtering a separate water sample through GFF filters with the filters frozen until analysis. Subsequently, filters were ground in 90% acetone and chlorophyll *a* concentration was quantified fluorometrically in extracts. Chlorophyll *a* constitutes approximately 1–2% of a microalgal cell (APHA, 1980) and the proportion of the suspended solids comprised of microalgae was estimated based on an average value of 1.5% chlorophyll *a* (mg) per unit of microalgae.

The community composition of the suspended solids was grouped into four broad categories: chlorophytes, cyanobacteria, diatoms and unknown microalgae. The relative proportion of each group was determined by categorising between 150 and 250 cells from 20 μL sub-samples using preserved material (1% Lugol's solution) at 1000× magnification under oil immersion (Leica DMLB light microscope).

Particle size distribution was determined with Malvern Mastersizer 2000 laser particle sizer using a 0.02–2000 μm lens (Malvern Instruments). The detector was calibrated and aligned before each batch of samples using water as the background correction.

2.4. Industrial harvest of suspended solids

Suspended solids were harvested from the water of each culture and settlement pond using a centrifugal algal harvesting system at the MBD Energy Research Facility, James Cook University, Townsville (Evodos BV – SPT325; serial number: 609805; operated at 3800 L hr^{-1} and ambient temperature). The post-harvest effluent water was collected from the harvesting of each of the culture and settlement ponds for analysis of remaining suspended solids (see Section 2.5.1). To ensure that sufficient biomass was harvested for the quantification of fatty acids and physico-chemical parameters of the paste, and the production and assessment of biochar, the biomass from all six samples (6 × 1000 L) from the culture ponds were combined as were all six samples (6 × 1000 L) from settlement ponds. This provided a single biomass paste of harvested suspended solids (paste) from each source. Once harvested, each paste was re-suspended in freshwater and re-processed through the centrifuge to ensure that any residual salt associated with the suspended solids was removed. Subsequently, three sub-samples (∼2 g each) from both the culture pond paste, and the settlement pond paste, were lyophilised (VirTis 2K) for analysis of fatty acids (see Section 2.5.2). The remaining paste was then spread on a Teflon baking tray and dried at 60 °C for three days. Once dried, a representative sub-sample was collected by dividing the dry paste 4–6 times using a riffle splitter. The sub-sample was homogenised using a SRM-standard ring mill (ROCKLABS Ltd.) to provide ∼20 g of ground sample for physico-chemical characterisation (see Section 2.5.3). The paste was packaged in a vacuum-sealed bag and stored frozen prior to the production of biochar (see Section 2.6).

2.5. Post-harvest analysis of suspended solids

2.5.1. Post-harvest analysis of effluent water

Post-harvest water was analysed for suspended solid concentration, particulate nitrogen, particulate carbon, chlorophyll *a*, microalgae community composition and particle size distribution (as above) to determine the quality and quantity of the suspended solids removed by the centrifuge.

2.5.2. Fatty acid analysis of harvested solids

Fatty acid analysis was conducted using lyophilised pastes. Direct *trans*-esterification was used to simultaneously extract and esterify the fatty acids from both samples, following methods described in Gosch et al. (2012). Gas chromatography was carried out in scan-mode on an Agilent 7890 GC (DB-23 capillary column) equipped with a flame ionisation detector (FID) for quantification and connected to an Agilent 5975C Electron Ionisation Turbo Mass Spectrometer (EI-MS) (Agilent Technologies Australia Pty Ltd) for identification of fatty acid methyl esters (FAME). Oven program and instrument settings followed David et al. (2002). The quantity of FAME was determined by comparison with FID peak areas of authentic standards (Sigma Aldrich), and corrected for recovery of internal standard. Total fatty acid content was calculated from the sum of all FAME.

2.5.3. Physico-chemical characterisation of harvested solids

The physico-chemical properties of the dried culture pond and settlement pond pastes were characterised prior to conversion to biochar. Loss on ignition (LOI) was used to estimate the organic and carbonate contents. LOI was calculated by combusting a small quantified amount of material (100–500 mg ± 0.1 mg) at 550 °C for 2 h, cooling the material to room temperature and weighing. Samples were subsequently re-combusted at 1000 °C for 1 h, cooled and re-weighed to determine the carbonate content (Heiri et al., 2001). Total nitrogen (TN) and total organic carbon (TOC) were determined following PN and PC methods as described above. Sulphur (S), phosphorus (P), iron (Fe), manganese (Mn), magnesium (Mg), potassium (K), calcium (Ca), and sodium (Na) were determined by inductively coupled plasma mass spectrophotometry (ICP-MS). Electrical conductivity (EC) and pH were determined in 10:1 water:sample mixtures according to Australian standard methods for soil analysis (Rayment et al., 1992). Cation exchange capacity (CEC) was determined using silver thiourea extracts (Rayment et al., 1992), and BET (Brunauer, Emmet, and Teller) surface area was determined by nitrogen adsorption (Particle and Surface Sciences Pty Ltd., in Gosford, New South Wales, Australia).

2.6. Biochar production and characterisation

Biochar was produced from both the dried culture pond paste, and the settlement pond paste, using slow pyrolysis under conditions previously optimised by Bird et al. (2011). Approximately 200 g of dried paste was weighed (to 3 decimal places), loaded into a wire mesh basket and suspended in a sealed 2 L stainless steel vessel inside a muffle furnace. The stainless steel vessel was constantly purged with dry nitrogen gas at 3.5 L min^{-1} and heated for over 1 h to a final hold temperature of 450 ± 5 °C (Bird et al., 2011). The furnace was maintained at 450 ± 5 °C for 2 h after which time the vessel was removed from the muffle. The resulting biochar was cooled to room temperature and weighed to determine weight loss accompanying pyrolysis. Subsequently, the physico-chemical properties (Section 2.5) were determined for both biochars.

2.7. Statistical analysis

The pre-harvest water quality characteristics and microalgal community composition data (the proportions of each group) were compared between pond types (culture and settlement) and within pond types (duplicates at three ponds nested within each pond type) in a nested design using multivariate permutational analysis of variance (PERMANOVA). Particle size distribution data was included as a single variable by selecting the size class that gave the maximum value (mode) for the particle volume distribution. The PERMANOVA was run on a Bray–Curtis similarity matrix using fourth root transformed data (PRIMER version 6 and PERMANOVA + version 1.0.4) (Anderson and M.J., 2008). A principal component analysis (PCA) plot was used to interpret the PERMANOVA results by relating the main vector loadings to both between and within pond type variation.

3. Results and discussion

3.1. Pre-harvest analysis of suspended solids

Despite the culture ponds and the settlement ponds being intrinsically linked through discharge of the former to the latter, the characteristics of the suspended solids from the two sources were significantly different (PERMANOVA; *Pseudo* $F = 10.28$, $P = 0.008$). There was also significant variability within pond types (PERMANOVA; *Pseudo* $F = 4.231$, $P = 0.002$). The concentration of suspended solids was 66% higher in the culture pond (131.8 ± 8.8 mg L^{-1}; $n = 3$) than in the settlement pond (87.6 ± 24.7 mg L^{-1}; $n = 3$), although one settlement pond (SP1) had notably higher TSS than the remaining two (Fig. 2A). The suspended solid results were reflected for all metrics as particulate nitrogen (Fig. 2B), particulate organic carbon (Fig. 2C) and chlorophyll *a* (Fig. 2D) concentration also tended to be higher in culture ponds, again with some variation within pond type. Conversely, the quality of the suspended solids, in terms of the content of particulate nitrogen, particulate organic carbon and chlorophyll *a* (as a percentage of suspended solid biomass), tended to be higher in the settlement pond (4.0 ± 0.8, 24.8 ± 4.7, 0.3 ± 0.0%, respectively; $n = 3$) compared to the culture pond (3.8 ± 0.6, 22.7 ± 3.1, 0.2 ± 0.1%, respectively; $n = 3$). The lower organic carbon content of culture pond suspended solids (22.7 ± 3.1%) compared to the settlement pond suspended solids (24.8 ± 4.7%) is likely explained by the presence of inorganic solids which are eroded from the floor of the culture pond by the movement of the aerators (Preston et al., 2001). These inorganic solids later settle in the settlement pond and are therefore not present in the suspended solids from settlement ponds. This implies that for the production of a high quality secondary product,

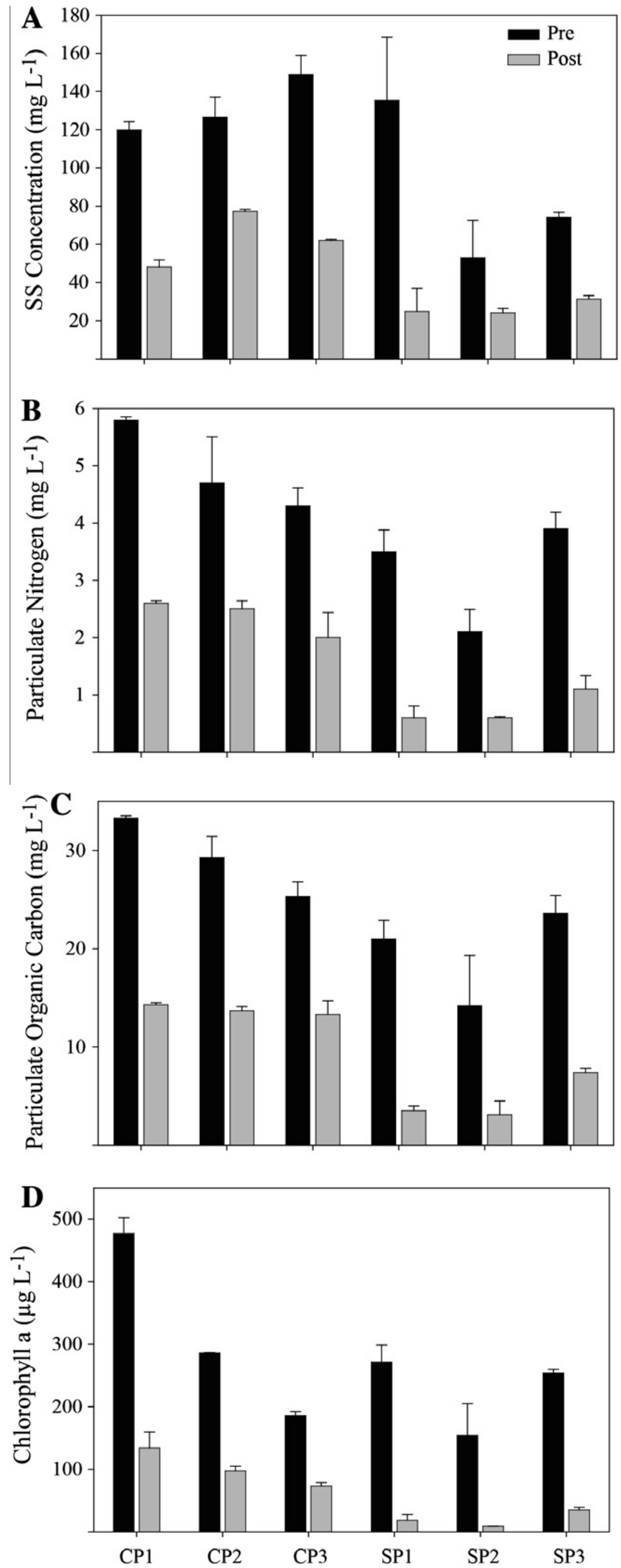

Fig. 2. Mean of (A) suspended solids (SS) (mg L^{-1}), (B) particulate nitrogen (mg L^{-1}), (C) particulate organic carbon (mg L^{-1}), and (D) chlorophyll *a* (µg L^{-1}) concentrations in discharge water from three prawn farm culture ponds (CP) and three prawn farm settlement ponds (SP) ($n = 2$). Black bars represent the mean concentration of each constituent before water has been processed in an Evodos centrifuge and grey bars represent mean concentrations after water has been processed.

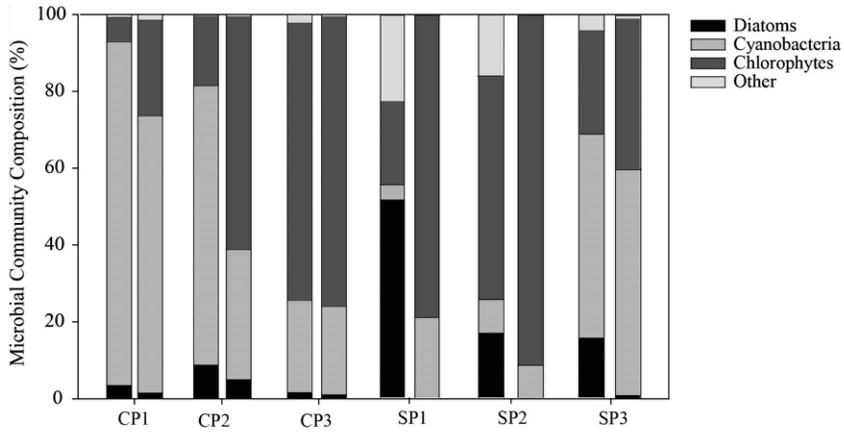

Fig. 3. Microbial community composition (% cells) of the suspended solids in discharge streams from the culture ponds (CP1, CP2 and CP3) and the settlement ponds (SP1, SP2 and SP3) from a commercial prawn farm. The first bar for each pond represents the community composition in the water pre-harvest and the second bar for each pond represents the community composition in the water post-harvest.

the harvest of waste suspended solids should occur from the stream discharging from the settlement ponds.

Using chlorophyll *a* as a proxy for microalgal biomass (Fig. 2D), it was estimated that 24.6 ± 7.8% (n = 3,) of the suspended solids in the culture pond water was microalgae compared to 27.5 ± 4.0% (n = 3, means of three ponds) of the suspended solids in the settlement pond. Filamentous cyanobacteria dominated in culture pond, making up between 24–89% of the microalgal community (Fig. 3) whereas diatoms (mainly centric *Cyclotella* spp. and *Chaetoceros* sp., and pennate *Cylindrotheca* sp.) were more common in the settlement pond, making up between 16–52% of the microalgal community (compared with 2–9% in culture pond) (Fig. 3).

Particle size ranged from a minimum of 0.04 μm up to a maximum of 563 μm across all ponds, and was more variable in the settlement pond water than in the culture pond water (Fig. 4A and B). The size fraction with the maximum value for particle volume distribution was 11–20 μm in both the culture pond and settlement pond water. This is in agreement with particle size distribution data in waste streams from recirculating farms where the majority of suspended solids are <30 μm (Cripps and Bergheim, 2000; Cripps, 1995). Given this similarity, suspended solids capture technologies such as rotating micro-screens, bead filters and flotation columns, which are used in advanced recirculating farms and are particularly efficient at capturing suspended solids >60 μm, could be adapted to flow-through land-based farms (Cripps and Bergheim, 2000). Cost effective technologies to harvest smaller biological solids (<60 μm) is a priority for the capture of energy-rich microalgae for bioproducts.

3.2. Post-harvest analysis of suspended solids

3.2.1. Post-harvest analysis of effluent water

The quality of water improved after processing through the Evodos (post-harvest) for both culture and settlement ponds (Fig. 2). Approximately 60% of the suspended solids were captured using centrifugal technology thereby reducing suspended solids in post-harvest water from a mean of 131.8 ± 8.8 mg L^{-1} to 62.5 ± 5.3 mg L^{-1} for culture ponds, and from 87.6 ± 24.7 mg L^{-1} to 26.7 ± 2.7 mg L^{-1} for settlement ponds (Fig. 2A). Particulate nitrogen (Fig. 2B), particulate organic carbon (Fig. 2C) and chlorophyll *a* (Fig. 2D) followed the same trend as the suspended solid concentration, with a lower concentration of each variable post-processing. There was also a significant effect of processing on the phytoplankton community (Fig. 3). In both the culture pond and settlement pond water, the community composition of post-

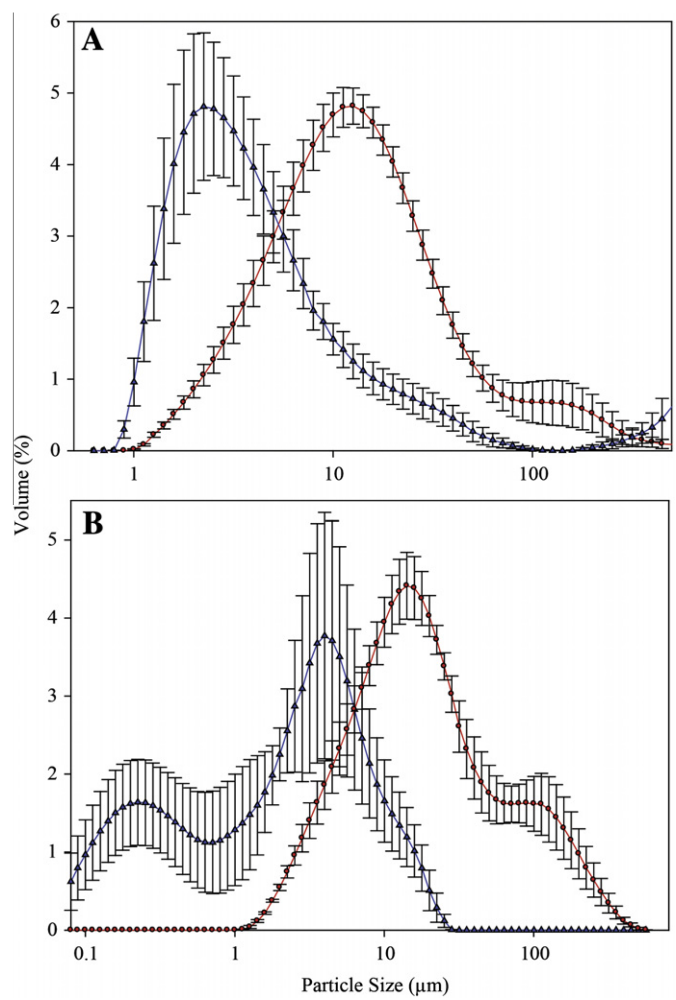

Fig. 4. Particle size distribution (logarithmic scale) of the suspended solids in the culture pond (A) and settlement pond (B) discharge water. Red lines represent the suspended solids in discharge water before it has been processed and blue lines represent the remaining suspended solids post-processing.

harvest water was dominated by small chlorophytes (1–5 μm) with almost 100% of the diatoms removed from the settlement pond water (Fig. 3). Given the high energy content and prevalence of long-chained fatty acids in diatoms, a capture rate approaching 100% is beneficial for the production of bioproducts from suspended solids. Furthermore, a 60% reduction in total suspended solid load provides opportunity for water re-use and improved water security for aquaculture.

The centrifuge selectively removed larger suspended solids, the specific targeting of diatoms and cyanobacteria over chlorophytes and fine sediment. This is demonstrated by a reduction in the proportion of the larger size fractions (>10 μm) in the suspended solids from water post-processing in both the culture pond (63 ± 5%) and the settlement pond (94 ± 1%) water (red line compared to blue lines; Fig. 4). Notably, settlement pond water has a fine-grained tail (particles < 1 μm) post-processing which is not present in culture pond water (Fig. 4B).

3.2.2. Fatty acid analysis of harvested solids

Microalgae are rich in fatty acids, including beneficial omega 3 (ω-3) and omega 6 (ω-6) polyunsaturated fatty acids which have applications in aquaculture feeds and nutraceuticals (Spolaore et al., 2006). Correspondingly, the harvested suspended solids from both the culture and settlement ponds were rich in fatty acids (measured as fatty acid methyl esters, FAME) with a mean total of 28.491 ± 0.257 and 41.990 ± 0.340 mg FAME g^{-1} DW suspended solids in the culture pond and settlement pond harvested solids, respectively (Table 1). The fatty acid profiles of the suspended solids harvested from both streams were similar and within each harvest the polyunsaturated fatty acid portion in the culture pond and settlement pond comprised 45.3% and 44.2% of the fatty acid profiles, respectively (Table 1). Furthermore, the harvested biomass from the culture ponds had 9.0% ω-6 and 23.5% ω-3 fatty acids, while the settlement ponds had 3.1% ω-6 and 26.6% ω-3 (Table 1). This is comparable to *Nannochloropsis* species which are specifically targeted for their polyunsaturated ω-3 and ω-6 fatty acids containing 5.8 ± 0.8% ω-6 and 20.9 ± 3.7 ω-3 (Huerlimann et al., 2010). Notably, the ω-6:ω-3 fatty acids ratios were low at 0.4 and 0.1 in the culture pond and settlement pond harvests, respectively (Table 1). This makes the fatty acid profile of the harvested biomass, particularly from the settlement pond, preferable for inclusion into nutraceuticals, because western diets are deficient in ω-3 fatty acids due to the industrial production of ω-6 rich cereal grains (Simopoulos, 2002). A higher proportion of DHA (3.9 ± 0.0 mg g^{-1}), an ω-3 fatty acid, occurs in the settlement pond harvest than in the culture pond harvest (0.8 ± 0.0 mg g^{-1}). Diatoms are rich in DHA (Huerlimann et al., 2010) and are therefore most likely the drivers of different ω-6:ω-3 ratios between culture and settlement pond harvested solids (Table 1).

Table 1
Fatty acid composition (mg FAME g^{-1} DW suspended solid) of waste harvested suspended solids collected from an aquaculture culture pond and settlement pond discharge. Data are mean (±1 SE).

Fatty acid	Grow-out	Settlement
C12:0	0.08 (0.00)	0.08 (0.00)
C14:0	1.74 (0.16)	2.81 (0.01)
C14:1	0.17 (0.01)	0.15 (0.01)
C15:0	0.20 (0.01)	0.28 (0.01)
C15:1	0.33 (0.04)	0.38 (0.01)
C16:0	6.49 (0.85)	8.04 (0.03)
C16:1 (7)	0.26 (0.04)	0.14 (0.00)
C16:1 (9)	3.79 (0.43)	3.63 (0.02)
C16:2 (7,10)	0.70 (0.15)	0.20 (0.00)
C16:2 (9,12)	0.59 (0.05)	1.74 (0.01)
C17:0	0.00 (0.00)	0.11 (0.01)
C17:1	0.62 (0.06)	0.76 (0.02)
C16:3 (6,9,12)	0.77 (0.14)	0.26 (0.01)
C16:4 (4,7,10,13)	0.70 (0.09)	0.85 (0.02)
C16:4 (6,9,12,15)	0.60 (0.25)	1.24 (0.04)
C18:0	0.29 (0.02)	0.39 (0.10)
C 18:1 (*cis* 9)	1.04 (0.17)	1.37 (0.01)
C 18:1(*trans* 9)	0.32 (0.03)	0.34 (0.00)
C 18:2 (9,12)	2.40 (0.49)	1.10 (0.01)
C18:3 (6,9,12)	0.18 (0.01)	0.24 (0.01)
C 18:3 (9,12,15)	2.82 (0.44)	2.32 (0.01)
C18:4 (6,9,12,15)	0.61 (0.05)	2.30 (0.02)
C20:0	0.10 (0.01)	0.24 (0.01)
C20:1	0.0 0 (0.00)	4.59 (0.12)
C20:3 (8,11,14)	0.19 (0.02)	0.05 (0.00)
C 20:4 (5,8,11,14)	0.17 (0.01)	0.22 (0.01)
C 20:5 (5,8,11,14,17)	2.42 (0.23)	4.13 (0.03)
C22:0	0.16 (0.01)	0.00 (0.00)
C24:0	0.00 (0.00)	0.15 (0.02)
C 22:6 (4,7,10,13,16,19)	0.76 (0.04)	3.89 (0.04)
Total	28.49 (0.26)	41.99 (0.34)
Total saturated	9.06 (0.71)	12.10 (0.89)
Total monounstaturated	6.52 (0.44)	11.35 (0.61)
Total polyunsaturated	12.91 (0.25)	18.54 (0.39)
ω-3	6.71 (0.55)	11.19 (0.76)
ω-6	2.57 (1.12)	1.32 (0.44)
ω-6/ω-3	0.38	0.12

3.2.3. Physico-chemical characterisation of harvested solids

The primary driver of differences in physico-chemical properties of harvest suspended solids (paste) between the two sources is the higher inorganic content of suspended solids harvested from the culture ponds. The ash content of the culture pond suspended solids was 44.5% compared to 35.2% in the settlement pond suspended solids (Table 2). Similarly, LOI at 500 °C, which is an indicator of organic content, and organic carbon were both lower in the culture pond suspended solids (51.4% and 25.9%, respectively) than in the settlement pond suspended solids (61.3% and 30.3%, respectively) (Table 2).

3.3. Biochar production and characterisation

Biochar produced from harvested suspended solids from the culture pond and settlement pond discharge streams reflect the physico-chemical properties of the solids in their low organic carbon content of 14.5 and 22.7%, respectively (Table 2). This is comparable to organic carbon found in macroalgal biochars (8.2–33.8%; Bird et al., 2011) and microalgal biochar (16%) Grierson et al., 2011. However, it is low relative to biochar based on ligno-cellulosic feedstocks, which range from 62 to 80% (Brewer et al., 2011).

The nitrogen content of the biochars (2.5% in the culture pond suspended solids and 3.5% in the settlement pond suspended solids; Table 2) were also within range reported for other algal biochars (1.6–5.3%) Bird et al., 2011 which is higher than biochar produced from municipal wastewater sludge (0.02–0.22%) (Hossain et al., 2010) and ligno-cellulosic sources (0.3% and 0.8%) (Brewer et al., 2011). The C:N ratio of the biochars produced from the suspended solids harvested from the culture ponds and settlement ponds was low (5.8 and 6.5, respectively; Table 2) even relative to microalgal biochar which had a ratio 10:1 (Grierson et al., 2011). This is reflective of the high nitrogen content in the harvested biomass (Table 2). This is a positive attribute, given that many agricultural systems are nitrogen limited.

The biochars from the harvested biomass are also rich in beneficial micronutrients, particularly K (2.0 and 1.4%), Mn (0.058 and 0.136%), Na (2.0 and 1.4%) and Fe (3.8 and 3.5%) in culture pond and settlement pond biochars, respectively (Table 2). It is important for plants to maintain neutral charge and balance nitrogen uptake, which occurs predominantly through uptake of the anion NO_3^-. Neutral charge is typically maintained through uptake of the cations K, P, and Ca (Chan et al., 2007). However, the biochars are also relatively high in the heavy metals, Cu (0.010% and 0.013%) and Ni (0.007% and 0.063%; Table 2), in culture pond and settlement pond biochars, respectively (Table 2). The nickel content of the settlement pond biochar exceeded levels deemed safe for nor-

Table 2
Composition of suspended solids and biochar which was collected from prawn farm culture pond and settlement pond discharge water. Note: "LOI" = loss on ignition; "CEC" = cation exchange capacity. Total and organic carbon and nitrogen data derived from non-acidified and acidified samples, respectively.

	Unit	Culture pond feedstock	Settlement pond feedstock	Culture pond biochar	Settlement pond biochar
LOI @ 500 °C	%	51.4	61.3	21.1	31.7
LOI @ 1000 °C	%	4.1	3.6	24.7	34.4
Pyrolysis loss	%	NA	NA	45.7	37.3
Ash	%	44.5	35.2	54.2	33.9
C:N		5.4	6.2	5.8	6.5
Organic carbon	%	25.9	30.3	14.5	22.7
Organic nitrogen	%	4.8	4.8	2.5	3.5
P	%	0.9	0.8	1.0	1.0
Ca	%	1.0	0.4	1.4	0.7
Mg	%	0.8	0.6	1.3	1.0
K[a]	%	1.3	0.9	2.0	1.4
Zn	mg kg^{-1}	164.0	185.0	235.0	274.0
Mn	mg kg^{-1}	386.0	861.0	575.0	1355.0
Na[a]	%	1.3	0.8	2.0	1.4
Fe	%	2.5	1.9	3.8	3.5
S	%	0.7	0.8	0.4	0.4
Cu	mg kg^{-1}	62.6	69.1	101.0	133.0
Pb	mg kg^{-1}	18.7	12.3	61.0	35.0
Ni	mg kg^{-1}	51.7	138.0	71.0	626.0
pH		6.2	5.9	7.9	7.2
BET surface area	$m^2 g^{-1}$	30.3	28.3	10.7	20.7
EC	mS cm^{-1}	5.9	3.3	8.9	6.9
Exchangable cations					
Ca	cmol (+) kg^{-1}	NA	NA	18.3	9.4
K[a]	cmol (+) kg^{-1}	NA	NA	13.9	12.2
Na[a]	cmol (+) kg^{-1}	NA	NA	58.8	36.6
Mg	cmol (+) kg^{-1}	NA	NA	10.7	6.0
CEC	cmol (+) kg^{-1}	NA	NA	3.5	0.5

[a] Potassium and sodium are only partially extracted with the acid digestion procedure used. All other element data are complete extractable values.

mal land application of biosolids (Ang and Sparks, 2000), although the stability and leaching of heavy metals from biochar is largely unknown and not considered in the biosolid application guidelines. A possible explanation for the high nickel content in the settlement pond biochar is the mobilisation of this heavy metal from acid sulphate soils regimes (Gröger et al., 2011) with the subsequent uptake into algal biomass (Saunders et al., 2012).

Cation exchange capacity (CEC) for the biochars was among the lowest of any values reported in the literature, with values of 3.5 and 0.5 cmol(+) kg^{-1}, in culture pond and settlement pond biochars, respectively (Table 2). The exchangeable cation, Na, is lower in this study (58.8 and 36.6 cmol(+) kg^{-1}; Table 2) than in Grierson et al. (2011) (110 cmol(+) kg^{-1}), which is likely attributed to the freshwater wash used which removes Na^+ and other exchangeable cations. Furthermore, the EC of the biochar is also relatively low at 8.9 and 6.9 mS cm^{-1} (Table 2) compared to biochar from other microalgae (Grierson et al., 2011).

The pH of biochar from culture pond and settlement pond harvests was 7.9 and 7.2, respectively (Table 2). pH of biochar ranges from 4–12 (Lehmann, 2007) and can be manipulated by adjusting pyrolysis conditions. The biochar in this study would aid in the stabilisation of soil pH to near neutral and would have applications for both acidic and alkaline soils.

Finally, BET surface areas are relatively low for the culture pond and settlement pond biochars, at 10.7 and 20.7 $m^{-2} g^{-1}$, respectively (Table 2). This is likely due to the high ash content and concentrated inorganic compounds which are hypothesised to block micro-pores and reduce the surface area of a biochar (Bruun et al., 2012). Accordingly, BET surface area (10.7 $m^{-2} g^{-1}$) was lower in biochar from the culture pond where ash content is high, with higher BET surface area (20.7 $m^{-2} g^{-1}$; Table 2) in the biochar from the settlement pond where ash content is lower. The low ash content of the biochar produced from the harvested suspended solids from the settlement pond (33.9%) is similar to that of other microalgal biochars (Grierson et al., 2011), while the higher ash content of culture pond biochar (54.2%) reflects a lower microalgal content and a higher content of inorganic particles which are eroded from the culture pond floor (Preston et al., 2001).

3.4. Bioresource scale

The scale of the bioresource of harvestable suspended solids from intensive land-based aquaculture of prawns is significant and will differ depending on the discharge stream from which suspended solids are harvested. For example, we estimate that a 100 Ha prawn farm releases more than 2 GL yr^{-1} (based on industry data; EPA monitoring; 2008–2009 grow-out season). The release of this water from the settlement ponds, from where water is discharged to the environment, would contain 2084 tonnes of suspended solids (based on a mean of 87.6 mg L^{-1}) (Fig. 2A). In this study ~70% of suspended solids from the settlement ponds are removed through harvesting (Fig. 2A) and therefore 1271 tonnes could be captured per annum from the settlement ponds. Utilising biochar as an example, 63% of the harvested suspended solid biomass can be converted to a valuable bioproduct (i.e. 481 tonnes from the settlement ponds), with the remainder being released through pyrolysis (Table 2), which can be used to generate energy (Panwar et al., 2012). The carbon and nitrogen contents of the settlement pond biochar were 22.7% and 3.5%, respectively. This equates to 109 tonnes of carbon and 17 tonnes of nitrogen being sequestered per annum. Finally, taking this approach to a larger scale, there are more than 900 Ha of intensive prawn aquaculture in Australia which could produce 8460 tonnes of biochar, sequestering 2034 tonnes of carbon and 252 tonnes of nitrogen. Expanding this simple but verifiable estimate to a global scale, where there are estimated to be more than 500,000 Ha of crustacean culture (FAO et al., 2010), the bioresource scales to 15,930,000 tonnes of harvestable solids, which at 63% efficiency would produce 10,035,900 tonnes of biochar, 2,278,149 tonnes of sequestered carbon and 351,256 tonnes of sequestered nitrogen. The extrapolation acknowledges caution given the variation in discharge concentration and load across farms and seasons (Jackson et al., 2004).

4. Conclusions

Harvestable suspended solids from intensive land-based aquaculture production offer a potential bioresource for conversion to bioproducts. The quantity and quality of fatty acids was higher in the biomass harvested from the settlement pond compared to the culture pond. However, both bioresources are rich in valuable ω-3 and ω-6 fatty acids. The harvested biomass is also an excellent bioresource for the production of biochar and potentially for other energy applications through conversions to biofuels (Biller and Ross, 2011). The point-source harvest of suspended solids from intensive aquaculture is an existing, large bioresource with potential to provide significant economic and environmental benefits. A comprehensive cost-benefit analysis of biochar production from prawn production or settlement ponds will add further rigour to these estimates.

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