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Growth comparison of microalgae in tubular photobioreactor and open pond for treating anaerobic digestion piggery effluent

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Abstract

The overwhelming interest in the use of microalgae to handle associated nutrient surge from anaerobic digestion technologies for the treatment of wastewater, is driven by the need for efficient nutrient recovery, greenhouse gas mitigation, wastewater treatment and biomass reuse. Here, the feasibility of growth and ammonium nitrogen removal rate of semi-continuous mixed microalgae culture in paddle wheel-driven raceway pond and helical tubular closed photobioreactor (Biocoil) for treating sand-filtered, undiluted anaerobic digestion piggery effluent (ADPE) was compared under outdoor climatic conditions between June and September 2015 austral winter season. Two Biocoils, (airlift and submersible centrifugal pump driven) were tested. Despite several attempts in using airlift-driven Biocoil (e.g. modification of the sparger design), no net microalgae growth was observed due to intense foaming and loss of culture. Initial ammonium nitrogen concentration in the Biocoil and pond was 893.03 ± 17.0 mg NH_4^+-N L^{-1}. Overall, similar average ammonium nitrogen removal rate

in Biocoil (24.6 ± 7.18 mg NH_4^+-N L^{-1} day^{-1}) and raceway pond (25.9 ± 8.6 mg NH_4^+-N L^{-1} day^{-1}) was achieved. The average volumetric biomass productivity of microalgae grown in the Biocoil (25.03 ± 0.24 mg AFDW L^{-1} day^{-1}) was 2.1 times higher than in raceway pond. While no significant differences were detected between the cultivation systems, the overall carbohydrate, lipid and protein contents of the consortium averaged 29.17 ± 3.22, 32.79 ± 3.26 and 23.29 ± 2.15% AFDW respectively, revealing its suitability as animal feed or potential biofuel feedstock. The consortium could be maintained in semi-continuous culture for more than three months without changes in the algal composition. Results indicated that microalgae consortium is suitable for simultaneous nutrient removal and biomass production from piggery effluent.

Keywords: Biocoil; Animal feed; Bioenergy; Ammonia; Wastewater

1. Introduction

The pig industry is the third largest producer of animal meat globally, with a population of 977.3 million pig heads [1]. Due to ballooning human population, this number will likely not decrease but be on an increase to ensure meat security for the increasing population, of which pig meat makes a significant contribution. However, owing to the nature of piggery operations and processing, large volumes of freshwater resources are consumed with concomitant generation of a significant amount of wastewater [2]. Maraseni and Maroulis [3] concluded that one pig produces 18 L of wastewater daily which corresponds to the sewage output of at least three persons. Poor piggery sewage management contributes significantly to climate change (carbon footprint) by emissions of greenhouse gases, nauseating odour, fly infestation, outbreak of diseases, pollution of soil, surface and ground waters by nutrient enrichment and leaching [3,4]. Hence, the sustainability of this industry depends on the management of the emerging environmental challenges posed by piggery operations.

A number of technologies commonly used for conventional wastewater treatment can be applied to mitigate the harmful effects of piggery wastewater on humans and the environment. These technologies include aerobic lagoons, oxidation ponds, anaerobic digestion, evaporative ponds,

facultative ponds, aquatic plants and constructed wetlands [3,5]. Anaerobic digestion provides a tremendous primary remedy for odour control, capturing of gases, degradation of organic matter and other toxic pollutants in the effluent in addition to treatment of large quantity of waste [6]. However, available conventional technologies cannot handle the associated nutrient surge that follows anaerobic biodegradation [6,7], and reduction of further emission of gases. Discharge of treated effluents with high nutrient concentrations can promote eutrophication of aquatic ecosystem and deterioration of both surface and ground waters [8,9]. At the heart of this problem is the need for maximum nutrient recovery and provision of clean water that could meet quality standards for typical piggery operation. Therefore, there is a need for a technology that maximizes nutrient recovery while mitigating greenhouse gases emission.

Remediation of wastewater by microalgae has become an increasingly important technology for nutrient recovery and greenhouse gas release mitigation from anaerobic digestion piggery effluent (ADPE). This approach is environmentally sound since it depends on the principle of natural ecosystems [10]. The issue of secondary pollution is solved due to very efficient biomass reuse and nutrient recycling. Moreover, the versatility of microalgae is further exploited in the production of biofertilizers, feed for animals and fine chemicals [10,11]. However, the macromolecular composition (lipids, carbohydrates, proteins, nucleic acids) and pigments contents of microalgae biomass are influenced by growth conditions.

Nutrient recovery from anaerobic digestion piggery effluent (ADPE) by microalgae has gained renewed interest over the last decade [2,4,12,13]. Several microalgae have been reported as good candidates for wastewater bioremediation including *Chlamydomonas* sp., *Euglena* sp., *Micractinium* sp., *Botryococcus* sp., *Coelastrum* sp., *Chlorella* sp., *Scenedesmus* sp., and *Oscillatoria* sp., (to mention a few) [13–17]. Among these microalgae species, *Chlorella* and *Scenedesmus* sp. appear to be the most robust and versatile due to tolerance to different wastewater conditions [6,10,15,18–23]. Ayre [24] reported a *Chlorella* sp., *Scenedesmus* sp. and a pennate diatom that can grow efficiently on undiluted ADPE with up to 1600 mg NH_4^+-NL^{-1}. These strains were selected after bioprospecting several microalgal strains potentially suitable for growth in undiluted ADPE.

Microalgae cultivation systems can be classified into open ponds and closed photobioreactors (PBRs). Due to simplicity and cost effectiveness in wastewater treatment, open ponds are mostly used [8]. However, less productivity and biotic pollution of undesired species [25] are challenges common with open pond systems. Furthermore, the dark nature of effluents hampers efficient light utilization in open ponds. Closed PBRs offer better regulation and control of physical and chemical factors [26]. Some of the attractive features of the closed PBRs include being less prone to biotic pollution, stable culture conditions, ability to control temperature and hydrodynamics and improved efficiency in light distribution [26,27]. The increase in surface area to volume ratio of closed PBRs would maximize light utilization by microalgae growing in wastewater thereby would influence nutrient removal and productivity positively especially in an effluent such as ADPE.

To the best of our knowledge, reports on the comparison of these two systems treating undiluted ADPE by microalgae are limited. Molinuevo-Salces et al. [28] compared the performance of open and closed (6 L) PBRs treating centrifuged and consequently diluted ADPE under laboratory conditions using microalgae-bacteria consortia and reported similarity in the removal of organic matter but different mechanisms of removal from both reactor configurations. In a similar investigation, Zhou et al. [29] used a semi-continuous method at the optimal hydraulic retention time of 72 h, cultivated a local isolate of microalgae (*Auxenochlorella protothecoides* UMN280) from a municipal wastewater treatment plant on autoclaved concentrated municipal wastewater with nutrient removal rates at 59.70% and 81.52% for total nitrogen and phosphorus respectively, using a 25 L Biocoil. However, comparison of open raceway pond and closed PBRs treating undiluted ADPE by microalgae under outdoor climatic conditions is yet to be reported. Hence, this first study was undertaken to test the feasibility of growing the microalgae consortium, *Chlorella* sp., *Scenedesmus* sp. and a pennate diatom, in a helical tubular closed PBR (Biocoil) using sand-filtered, undiluted ADPE. The microalgae growth, productivity, biochemical composition and ammonium removal rate under this closed PBR cultivation system was compared with that of the open raceway pond cultivation system under outdoor climatic conditions of Western Australia during the winter season.

2. Materials and methods

2.1. Microalgae culture

The microalgae consortium used in the current study were *Chlorella* sp., *Scenedesmus* sp. and pennate diatom isolated previously from ADPE [24]. The isolates were pre-acclimated to high ammonia [24]. The microalgae were first grown in batch phase using both cultivation systems [32]. Following this phase, both cultivation systems were switched to semi-continuous operations with the Biocoil as determinant for culture harvest on attainment of maximum cell density [32].

2.2. Anaerobic digestion of piggery effluent (ADPE) and growth media

The ADPE was collected from a covered anaerobic facility at Medina Research Station, Kwinana, Western Australia (32°13′16″S, 115°48′30″E). The research facility employs anaerobic digestion pond to treat its wastewater [24]. The ADPE contains high nutrient (e.g. nitrogen and phosphorus) content at the point of discharge to the evaporation pond [24]. The effluent was sand-filtered into a1000 L tank and used with no further treatment for algal cultivation. The ADPE storage tank was protected from sunlight. The chemical composition of the medium was partially characterised by Ayre [24].

2.3. Experimental setup and cultivation conditions

The cultivation systems consisted of an open raceway pond and two helical tubular

(Biocoil, Fig. 2a–d) closed PBRs [30]. The paddle wheel-driven raceway pond was operated at a working volume of 160 L and liquid velocity of 22 cm s^{-1}[31]. Biocoils were helical tubular PBRs with two different mixing designs. Both designs consisted of a non-toxic clear vinyl tubing (food-grade, internal diameter, 25 mm; external diameter, 30 mm) coiled around a steel mesh frame (Fig. 2d). The steel mesh frame is 0.9 m high and has a diameter of 70 cm [26]. One of the Biocoils was driven by an airlift system [26]. A submersible centrifugal pump (PU4500, Pond Max, 4500 L/h) housed in a 20-L dark plastic container was used for generating mixing in the second Biocoil (Fig. 2c). The pump-driven Biocoil has a total volume of 40 L and a flow rate of 40.3 cm s^{-1} in the coil.

We investigated two airlift/downcomer geometry of the airlift system (Fig. 2a, b; see designs II and IV in [26]). Due to inability to grow the consortium in the airlift-driven system, the pump-driven Biocoil (henceforth referred to as Biocoil) and raceway pond were continued and compared. An evaporative passive cooling system (operated between 10:30 am and at 4:30 pm) was used for keeping the coil temperature under 25 °C. The effluent inoculum ratio ranged from 40 to 60% while partial harvest at semi-continuous was carried out at 25–50% [32]. Before sampling, tap water was added to the raceway pond to replenish evaporation loss. Daily ten- minutes interval recording of solar irradiance and rainfall for the period of the experiment (June – September) was downloaded from Murdoch University Weather Station (http://wwwmet.murdoch.edu.au).

2.4. Analytical methods

In both cultivation systems, cell count and medium ammonium nitrogen concentration were determined by collecting samples at 10:30 am every second day. Biomass concentration (AFDW, Ash-free dry weight), biochemical composition (total protein, carbohydrate, and lipids) and chlorophyll contents of the biomass were assayed fortnightly. Filter papers that contained the filtered microalgae were stored after filtration and washing by folding in two and blotted gently to remove any excess water. The filter papers were placed in small plastic bags in a closed container and stored at − 20 °C in the dark until extraction and analysis.

Cell count was carried out using an improved Neubauer chamber [32]. Ash-free dry weight (AFDW, mg L^{-1}), total lipid content, total carbohydrate, total protein, and chlorophyll contents were measured according to the method of Moheimani et al. [32].

2.5. Operational condition

Flow rates in the cultivation systems were determined using the tracer method with 1 M HCl [26,31]. The sand-filtered effluent was partially characterised for ammonia, dissolved oxygen, and pH. The temperature in the Biocoil was tracked with an underwater data recorder (Tinytag TG-4100) while DO and pH were monitored manually by daily measurements at 9 am, 12 pm, 3 pm, and 5 pm. YSI 6-

Series multi-parameter Sondes were used to monitor the DO, temperature, and pH [24] in situ. Measurement of ammonia was carried out using a photometer (Spectroquant Move 100, HC553485).

2.6. Statistical analysis

Measurements of ash-free dry weight (AFDW), chlorophyll, and biochemical components were done in triplicates. Values were expressed as means with standard errors. A t-test was used to determine significant differences between various parameters of microalgae content in cultivation systems. A One-way Repeated Measures ANOVA was used to compare significant differences among the various microalgae cell density in both cultivation systems.

3. Results and discussion

3.1. Growth of algal consortium

The experiment was conducted in the austral winter season between 01 June and 25 September 2015. At the commencement of the experiment, the cultures in both raceway pond and Biocoil were operated as a batch culture in order to identify the optimum and suitable cell densities for semi-continuous operation (Fig. 1E and F). The cell density in both systems declined two days after inoculation and thereafter showed a slow increase between days 3 and 16 (Fig. 1E and F). At the stationary phase of the batch culture (day 16), maximum *Chlorella* densities of 51.8×10^6 cells mL^{-1} and 113.5×10^6 cells mL^{-1} were respectively attained in raceway pond and Biocoil (Fig. 1E and F). In both cultivation systems, *Chlorella* sp. remained the dominant species (One-way repeated measures ANOVA, $F_{pond} = 337.65$, $F_{biocoil} = 137.47$, $P < 0.001$) Previous research found the *Chlorella* dominating the ADPE grown culture for wastewater treatment [24]. The abundance of the consortium in both cultivation systems was ranked as *Chlorella* > *Scenedesmus* > Pennate diatom.

The second most dominant alga in both cultivation systems was *Scenedesmus* sp. (One-way repeated measures ANOVA, $P < 0.001$). The overall growth of *Scenedesmus* sp. during the batch phase in the

Biocoil showed a decrease on day 2 and gradual increase between days 3 and 6 (Fig. 1F). Between days 7 and 16, there was a decrease but unsteady fluctuation in the *Scenedesmus* cell density in the Biocoil. However, the growth of this species in the pond was almost steady between days 1 and 5 probably due to rainfall on days 2, 3 and 4 (Fig. 1E). A decrease in *Scenedesmus* cell density was observed after day 7, nevertheless; it showed an exponential increase beginning from day 10 until the stationary phase. *Scenedesmus* sp. showed dominance and better growth in the raceway pond than Biocoil (Fig. 1E and F). The difference in growth pattern between the two systems was likely due to the shear stress from the mixing apparatus, which is more significant in the Biocoil compared to the gentle mixing paddle wheel in the pond. This finding was confirmed by microscopic observation which showed that the cell morphology of *Scenedesmus* cells appeared broken and separated in the Biocoil but remained intact in the raceway pond.

Several attempts to grow the microalgae consortium in airlift driven Biocoil were not successful (Fig. 1D). Although two airlift geometries were tested (Fig. 2a and b), the cell densities were observed to decline after each trial (Fig. 1D). The major challenge of the airlift design IV was intense foaming of culture that led to a continual overflow and loss of a copious amount of the culture (up to 3 L day^{-1}). This problem could not be eliminated even by changing the sparger design. The airlift design II minimized loss of culture, however, intense foaming was not eliminated probably due to the turbulence created in the airlift, and the microalgae flocculated with the foam and stuck to the sides of the airlift riser. The foaming of culture and the sticking of cells to the photostage coil were identified as major problems of the airlift system [26]. For instance, Moheimani et al. [26] trialled the feasibility of growth of three species of coccolithophorid algae (*Pleurochrysis carterae*, *Emiliana huxleyi* and *Gephyrocapsa oceania*) in airlift-driven Biocoil and reported the inability of the algae to grow in the system. The failure was attributed to cell damage by high shear and bubble effects from the airlift flow regime. However, Raes et al. [31] successfully grew a halophilic green alga *Tetraselmis* sp. MUR-233 in airlift-driven Biocoil and reported that biomass productivity of 85 mg AFDW L^{-1} day^{-1} and reliable semi-continuous operation were achieved in three months with addition of CO_2 at controlled pH of 7.5. Similarly, Zhou et al. (2012) using a semi-continuous method

at three-day hydraulic retention time, grew a facultative heterotrophic freshwater microalga, *Auxenochlorella protothecoides* UMN280 on an autoclaved concentrated municipal wastewater in a 25-L Biocoil with a net biomass productivity of 1.51 g L^{-1} day^{-1}. Hence, successful growth of microalgae in airlift-driven Biocoil appears to be species specific.

At the onset of semi-continuous operation, both cultivation systems were harvested at 50% and replaced with required quantity of sand-filtered ADPE. The maximum cell densities achieved in June for the semi-continuous cultures for *Chlorella* were 147.8 × 10^6 cells mL^{-1} and 57.1 × 10^6 cells mL^{-1} in the Biocoil and pond respectively after 12 days. There were fluctuations in *Scenedesmus* density in both systems. It is important to note that the density of *Scenedesmus* in the raceway pond was 3.3 times higher than that in Biocoil (Fig. 1E and F), a trend similar to the batch phase. On the 9 July, the semi-continuous operation was affected by low temperature. Following freezing (Fig. 2e) and crashing of the culture in the Biocoil, 50% of the culture in both systems were removed and replaced with fresh inoculum. Due to a sudden decrease in cell density (Fig. 1E and F) and subsequent increase in cyanobacteria density (data not shown) in the Biocoil, the cultures were terminated on 25 July 2015. It is important to note that before the 25th of July, the concentration of cyanobacteria remained negligibly small as indicated by the negligible values of chlorophylls c1 + c2, phycocyanin, and phycoerythrin.

Consequently, both cultivation systems were restarted on 1 August 2015 with an initial cell concentration of 13.1 × 10^6 cells mL^{-1} in both systems. This inoculum is approximately half the size used in batch phase because it was obtained from a 10-m^2 raceway pond used as finishing pond for the effluent treatment. The *Chlorella* sp. grew to 90 × 10^6 cells mL^{-1}in the Biocoil and 51.5 × 10^6 cells mL^{-1} in the pond and 40% was harvested and replenished with the same quantity of effluent on 11 August (day 11). As the growth of microalgae decreased after 13 August in the Biocoil, biofilm formed on the tubes wall was observed. The addition of 15 mL, 0.01%*w*/*v* sodium bicarbonate (1.2 mM $NaHCO_3$) on 14 and 15 August increased the density of *Chlorella* from 25.7 × 10^6 to 148.3 × 10^6 cells mL^{-1} between 14 and 19 (day 19) August for Biocoil. However, the addition of a proportional amount of sodium bicarbonate to the pond had little effect on the cell density (Fig. 1E

and F). Similarly, the *Scenedesmus* density in the Biocoil increased from 2×10^4 cells mL^{-1} to 34×10^4 cells mL^{-1} in seven days while an increase in the pond over the same period was lower (1×10^4 cells mL^{-1} to 8×10^4 cells mL^{-1}). Interestingly, the increase in *Scenedesmus* density in the Biocoil over this period was approximately five times the increase in the pond. Diatom growth was similar in both cultivation systems during this period. In general, diatom density showed dominance in the pond compared to the Biocoil (Fig. 1E and F). The less noticeable increase in the raceway pond's cell densities over this period would be attributed to dilution effect from rainfall (Fig. 1A). On 24 August (day 24), 40% of the cultures in both systems were harvested. Failure of the evaporative cooling system to function on 25 August resulted in temperature increase which would be responsible for declined cell density observed after this day in the Biocoil. On 5 September (day 36), 25% of cultures in Biocoil cultivation system was harvested and replaced with effluent. The cell densities in both systems at this point were almost the same. However, the pond showed a decrease in cell numbers of *Chlorella* sp. probably due to the increase in protozoa density (Fig. 1E). Therefore, 50% of the culture in both systems were removed and replaced with the inoculum on the 14 September (day 45). The increase in protozoan density observed in the pond necessitated monitoring of its trend in both systems. Protozoa reduction rate in the Biocoil (14.5%day^{-1}) was 2.4 times higher than the raceway pond. An increase in protozoan level in the pond (20, 25, 27, 35×10^4 cells mL^{-1} on 5, 11, 23 and 25 September 2015 respectively) was noticeable in many days when compared to the number in Biocoil (6, 11, 18, 2×10^4 cells mL^{-1}) on the same days (Fig. 1E and F).

Efficient turbulent mixing systems move microalgae through different light quality and quantity and ensure that they spend a longer time in the illuminated areas of the cultivation systems. Research has shown that high turbulence increases the rate of exchange of nutrients and products between the cells and medium with a direct relationship with productivities [33]. However, shear stress resulting from increased turbulence, the action of mechanical pumps, eddies in the growth medium, air bubbles, and high liquid speed can have a damaging effect on microalgae cells [26,31,34,35]. Although the consortium used was able to withstand the damaging effect of the mechanical pump, the more fragile *Scenedesmus* and pennate diatom species grew better in the open pond over the long term

(Mean, *t*-test, $P < 0.05$; Mann-Whitney test, $P = 0.021$). These cells were relatively bigger in size than the *Chlorella* sp. as observed under the microscope. *Scenedesmus* sp. usually occurs as quadruplets or more, but single cells of this microalga were consistently observed in the Biocoil compared to the open ponds throughout the cultivation period. This separation of the cells in the Biocoil would be as a result of the effect of the mechanical action of the pump. In 1-m^2raceway pond, turbulent mixing system is achieved in the first 3-m and the rest, a laminar flow [31], the gentle mixing action of the paddle wheel system would explain the increased density of protozoa compared to the Biocoil (Mann-Whitney test, $P = 0.005$). It is necessary to mention that the raceway pond is open to the atmosphere, and this would also affect protozoa dynamics and diversity of the culture.

3.2. Biomass productivity and biochemical composition

Biochemical compositions of the grown algae are summarised in Table 1. The highest monthly volumetric productivities for the pond (24 ± 0.13 mg AFDW L^{-1} day^{-1}) and Biocoil (47 ± 0.13 mg AFDW L^{-1} day^{-1}) were attained in August. Average biomass productivity in the Biocoil (25.03 ± 0.24 mg AFDW L^{-1} day^{-1}) was 2.14 times higher than the productivity in the raceway pond. Rainfall (as noted earlier) may have had a dilution effect on the pond resulting in low biomass productivity (Fig. 1A). Overall, protein, carbohydrate and lipid contents of consortium biomass were 23.29%, 29.17%, and 32.79% respectively

Both cultivation systems had relatively higher lipid content than carbohydrate and proteins. Higher biomass concentrations observed in the Biocoil may have contributed to the difference between the two systems. However, temperature, light quality, and quantity are known to change the overall biochemical contents of many microalgae [36]. While temperature exerts its effects on microalgal composition and biochemical reactions to alter the biochemical composition of the cell, light is used for biosynthesis of carbon compounds [37]. It has been reported that microalgae accumulate lipids under stress conditions. It is also known that organisms (e.g. microalgae) maintain their structural integrity and membrane fluidity with the help of lipids [38]. Singh et al. [39] investigated the potential of cultivating mixotrophic microalgae on digested poultry effluent and reported maximum biomass productivity of 0.076 g L^{-1} day^{-1} and lipid, protein and carbohydrate contents of < 10%, 39%, and

22% respectively. These authors concluded that the biomass could potentially be used as feed supplement for animals. Contrary to this result, our study showed that the consortium had higher lipid content (32.79% AFDW) compared to carbohydrate and proteins in both cultivation systems. Hence, the biomass of this consortium could potentially serve as a source of animal feed or biofuel feedstock. Obviously further studies are necessary to analyse the quality of the biomass as a source of animal feed.

Furthermore, for highly productive microalgae cultivation systems, the cost of the production of biofuel stands at 77%, 12%, and 7.9% on the tripod of growth, harvesting and lipid extraction respectively [40]. It is suggested that the quest for the production of biofuel from microalgae will make sense if coupled with wastewater treatment. Excitingly, our data show that although this microalgae consortium can grow in high ammonia piggery effluent and remove nutrients (25.25 ± 7.89 mg L^{-1} day^{-1}), they can also have high lipid content, hence, striking a balance for simultaneous nutrient removal and lipid production in effluent wastewater. Similar findings on the cultivation of *Chlorella vulgaris* in synthetic wastewater that produced 20–42% lipids (on dry weight basis) with nutrient removal efficiencies of 96 and 97% for total phosphorus and ammonium, respectively, has been reported [41].

3.3. Chlorophyll composition

Chlorophyll measurement revealed the presence of a significantly higher amount of *chl-a* relative to *chl-b* in both systems (*t*-test, $P < 0.05$, Table 1). Chlorophylls-*a* and *b* concentrations ranged between 1.46 ± 0.98–6.15 ± 01.5 mg L^{-1} and 0.46 ± 0.06–1.24 ± 0.34 mg L^{-1}respectively in the Biocoil; while chlorophyll $c_1 + c_2$ values were approximately zero (Table 1). Chlorophyll-a content of cells in both cultivation systems increased with increase in biomass concentration (Biocoil, $r^2 = 0.983$, $P = 0.119$; Pond, $r^2 = 0.995$, $P = 0.0658$). The low chlorophylls-*a* and *b* obtained in both systems during the month of September correspond with low biomass productivity (Table 1). Overall, the chlorophyll-*a* content of the biomass obtained from the Biocoil (3.76 ± 1.20 mg L^{-1}) was 2.7 times higher than that obtained in the raceway pond. The prevalence of *chl-a* and *chl-b* relative to *chl-*

c indicate the dominance of green microalgae, *Chlorella*, *Scenedesmus* sp. [42], throughout the experimental period.

3.4. Effluent ammonium nitrogen removal

The ammonium trends in both cultivation systems are summarised in Fig. 1H. The ammonia concentration of the sand-filtered effluent was 1232.39 ± 54.28 mg $NH_4 + -$ N L^{-1}. The initial ammonium concentration in the pond and Biocoil systems was 893.03 ± 17.00 mg NH_4^+-N L^{-1} for the batch phase, after mixing with the inoculum. During the batch phase, the ammonium removal in the pond (22.19 mg L^{-1} d^{-1}) was 1.44 times higher than the Biocoil for a period of 16 days, even when biomass concentration was higher in Biocoil. The difference in the ammonium removal rate between the two systems could be due to better stripping of ammonia in open ponds compared to closed photobioreactors. In other words, under the experimental conditions, ammonia removal in open pond was not purely biological. The overall removal rates of ammonium in both cultivation systems during the semi-continuous operation were similar, 25.97 ± 5.37 and 24.06 ± 9.48 mg NH_4^+-N L^{-1} for raceway pond and Biocoil, respectively. This could be because open pond can reduce ammonia easier compared to the closed PBRs. The highest monthly ammonium removal rates for the pond (30.9 ± 10.1 mg NH_4^+-N L^{-1} day^{-1}) and Biocoil (39.2 ± 9.5 mg NH_4^+-N L^{-1} day^{-1}) were achieved in July and August respectively while the lowest were achieved in September in both systems (Table 1) due to low cell density. Ammonium removal rate in both systems is directly related with biomass productivity (Biocoil, $r^2 = 0.963$, $P = 0.175$; Pond, $r^2 = -0.264$, $P > 0.05$) which is directly linked with chlorophyll content. As stated earlier, Zhou et al. 2012 grew *Auxenochlorella* sp. in autoclaved concentrated municipal wastewater using a 25 L Biocoil and achieved a removal efficiency of 26 mg L^{-1} day^{-1} for total nitrogen in three days. In the investigation by Molinuevo-Salces et al. [28], algae grown in both open and closed systems removed all effluent ammonium. However, we did not observe similar findings of ammonium removal in this study. Ammonium was reasonably removed in both cultivation systems though higher removal was achieved in the open pond. Molinuevo-Salces et al. [28] reported that in addition to nitrification and denitrification processes, ammonia stripping is the main driving force for ammonia removal in open ponds.

Reports have shown that microalgae can metabolize inorganic nitrogen in wastewater such as ammonium [7,29,43]. Ammonium nitrogen is the dominant nitrogen species accounting for approximately 90% of total nitrogen in sand-filtered ADPE. Previous investigations have revealed that high concentration of ammonia is toxic to microalgae [20,44]. Furthermore, at pH > 8, ammonium is considered an inhibitory compound [28]. However, the ability of our current microalgae consortium to survive in growth media with initial ammonia concentration up to 910 mg NH_4^+-N L^{-1} in a closed and open cultivation systems clearly illustrate that these species are robust and can not only tolerate but grow well at high ammonia concentration [24]. Removal of ammonium nitrogen from wastewater is either by direct ammonia uptake by microalgae or ammonia stripping [15,45]. Reports have shown that ammonia stripping occurs under conditions of elevated temperatures (> 20 °C),high concentration of urea and alkalinity [15,28]. Although the pH of both cultivation systems were usually above 8 and, considering that the experiment was conducted in winter with average temperature of the reactors, not > 20 °C, it could be stated that removal of ammonium nitrogen from the effluent was largely due to microalgae uptake and ammonia stripping process might not have had any significant contribution. It has been reported that the microalgae consortium is preferred to monocultures in wastewater treatment because single microalgal strains find it difficult to remove all the nutrients simultaneously from wastewaters due to the chemical complexity of wastewaters [7].

3.5. Physicochemical parameters

3.5.1. Changes in environmental variables

Successful cultivation and application of microalgae technology involve efficient control of physical, chemical and biological factors that influence the growth of microalgae [31]. Among these factors, light and temperature constitute the most limiting and critical indices for microalgal culture [46]. During the experimental period, the daily solar radiation ranged from 25.92 in June to 292.34 W m^{-2} in September with overall average of 159.02 ± 5.78 W m^{-2}. The lowest solar radiation was observed in July, which corresponds to the wettest and coldest month (Fig. 1A). The total rainfall for July was 117.5 mm (Fig. 1A) with a daily average of 3.79 ± 1.39 mm. Although June had scanty

rainfall, it is probably the cloudiest month due to its low sunshine radiation (Fig. 1A). The ammonium removal rate and productivity in the Biocoil for the month of July were 15.5 ± 3.6 mg NH_4-N L^{-1} $day^{-1}$and 20 ± 0.35 mg AFDW L^{-1} day^{-1} respectively. These values are lower than 39.2 ± 9.5 mg NH_4-N L^{-1} day^{-1} and 47 ± 0.13 mg AFDW L^{-1} day^{-1} in the same system for the month of August. Similar trend was observed in biomass productivity of the consortium in raceway pond (Table 1).

Light delivery, distribution and utilization are critical parameters for the design of microalgae cultivation systems due to the photosynthetic behaviour of microalgae [47]. Utilization of photosynthetic active radiation (PAR) by microalgae in outdoor cultures is affected by (a) seasonality (diurnal irradiance, variable cloudiness), (b) geographical location and latitude (solar elevation from sunrise to sunset), (c) geometry of cultivation systems design, (d) rate at which culture is diluted and (e) higher light scattering and diminution in turbulent flows [31,48,49]. Furthermore, high turbidity, high suspended particulate matters and dark colour of effluents are additional variables that influence light harvesting by microalgae in wastewater treatment [23,50]. A characteristic strength of the Biocoil system is that it is self- supporting arising from the coiled nature of the structure. The coiling of the Biocoil is advantageous in setting up relatively lengthy tubes in a small surface area [31]. The configuration of Biocoil creates a large surface area to volume ratio, which is ten times higher than the paddle wheel driven raceway pond with significant improvement on light conditions. This improvement on light distribution in the Biocoil is obvious from its higher volumetric productivity and daily nutrient removal than the pond, especially on days with lower sunshine. The higher volumetric productivity achieved in the Biocoil demonstrates that closed PBRs are less susceptible to negative effects of meteorological conditions in the austral winter season. This is because the Biocoil warms up faster than the open raceway pond (due to large surface area to volume ratio) and rapidly attains optimum temperature condition for photosynthesis to begin. The Biocoil system has shorter light path compared to the raceway pond, which accounts for higher biomass concentration, although would result to oxygen build-up in the coil, which is detrimental to microalgae by inhibition of photosynthesis. Removal of oxygen build-up from closed microalgae cultivation systems is a critical

engineering challenge limiting microalgae productivity in closed PBRs. However, the symbiosis between microalgae-bacteria consortia during wastewater treatment would reduce the harmful effect of oxygen accumulation in microalgae in the reactors.

The maximum temperatures in the pond and Biocoil were respectively 25.32 °C and 36.71 °C (Fig. 1B and C). On the 25 August (day 25 of semi-continuous), the Biocoil cooling system failed to function. This caused the temperature to hit high (Fig. 1E) and presumably, resulted to decline in microalgae growth. The daily minimum temperatures recorded for pond was 2.31 °C and − 0.4 °C for Biocoil (Fig. 1B and D). This low temperature in the Biocoil caused the system to freeze (Fig. 2a) and therefore crashed the cultures. This could be due to small culture volume compared to surface areas of the Biocoils as such phenomenon was not observed in the pond with higher mass to surface area. The introduction of heaters set at maximum temperature of 18 °C and operated between 8 pm and 6 am in both systems provided buffering capacity as minimum temperature in the systems was not below 3 °C afterwards (Fig. 1B and C). An earlier report [24] has indicated that this consortium could tolerate extremes of temperature (5–40 °C).

High and low temperatures above or below the tolerance limits of microalgae affect its performance. Passive evaporative cooling system is used in closed PBRs for temperature control. The challenge of this system is on its economics and sustainability due to fresh water limitation. Open raceway ponds do not need evaporative cooling system but still require fresh water addition to compensate for water loss by evaporation. Part of the solution to this engineering challenge could be the development of novel closed PBRs that do not require cooling system. Vadiveloo et al. [37] reported a novel flat-plate PBR that could allow passage of 50% of PAR while effectively blocking and capturing > 90% of infrared (IR) and ultraviolet (UV) radiations. IR and UV are responsible for temperature increase and damage to cells through mutation and death, respectively [37]. The captured radiations through integration with photovoltaics can be used to generate electricity. This electricity can be used to power generators, provide additional lighting or keeping the temperature constant by heating of the culture at night during winter.

The pH of the Biocoil and raceway pond averaged 8.5 ± 0.06 and 6.7 ± 0.008, respectively (Fig. 1B and C). The pH of the growth medium was 8.62 ± 0.13. It was observed that the daily pH of the pond tends to stabilize at a pH below 7 (Fig. 1B) while that of the Biocoil at pH around 9 (Fig. 1C). Our data demonstrated that the microalgae consortium used in this study could tolerate high pH as earlier asserted by [24], though a negative point in this regard since it results to ammonia stripping [8,28]. The ability of this consortium to tolerate high pH means that pH variation would not have been a limiting factor in our study. The attachment of microalgae to the walls of coil and subsequent improvement of growth and productivity on addition of sodium bicarbonate shows that carbon availability could have been a limiting factor. One potential solution to carbon limitation in wastewater-grown microalgae apart from external carbon addition could be to cultivate the microalgae in continuous or semi-continuous culture systems. The medium DO in raceway pond appeared to increase a little after inoculation but decreased progressively and remained fairly constant below 6 mg O_2 L^{-1} (Fig. 1G). Similarly, the overall medium DO in Biocoil remained around 9 mg O_2 L^{-1} throughout the growth (Fig. 1G). While changes in DO is due to metabolism by microorganisms, low DO as observed in the raceway pond favours nitrification as a means of ammonia removal [28].

4. Conclusion

Medium ammonia concentration above 34 mg NH_4-N L^{-1} is shown be toxic to microalgae (e.g. *Scenedesmus*) [52]. We successfully grew this microalgae consortium (*Chlorella* sp., *Scenedesmus* sp. and pennate diatom) in the Biocoil using sand-filtered high ammonium ADPE in austral winter season without change in algal composition. Although the overall ammonium removal rate was similar in open pond (25.9 ± 8.6 mg NH_4-N L^{-1} day^{-1}) and Biocoil (24.6 ± 7.18 mg NH_4-N L^{-1} day^{-1}), the biomass production was significantly higher (2.1 fold) in Biocoil than open raceway pond. The produced biomass could be best suited as a source of animal feed or bioenergy (i.e. bio-methane). There is no doubt that the further studies are required for

optimisation and also decision on the best cultivation system for treating anaerobic digestion piggery effluent. However, our promising results indicates the potential to treat this wastewater with extremely high ammonium using microalgae with no to very little dilution with freshwater.

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Fig. 1. *Panel A*, average solar irradiation (*solid line*) and total rainfall (*dotted line*), *panel B*, pond temperature (*solid line*) and pH (*dotted line*), *panel C*, pump-driven Biocoil temperature (*solid line*) and pH (*dotted line*), *Panel D*, temperature (*solid line*) and log transformed cell densities for *Chlorella* sp. (*dotted line*) and *Scenedesmus* sp. (*dotted-solid line*) for airlift-driven Biocoil, *panel E*, log-transformed cell densities for *Chlorella* sp. (*solid line*) and *Scenedesmus* sp. (*dotted line*) and pennate diatom (*dotted-solid line*) and protozoa (*solid broken-line*) for raceway pond, *panel F*, log-transformed cell densities of *Chlorella* sp. (*solid line*) and *Scenedesmus* sp. (*dotted line*) and pennate diatom (*broken-solid line*) and protozoa (*dotted-solid. Line*) for pump-driven Biocoil, *panel G*, dissolved oxygen for Biocoil (*dotted line*) and raceway pond (*solid line*), *panel H*, ammonium trend for Biocoil (*solid line*) and raceway pond (*dotted line*).

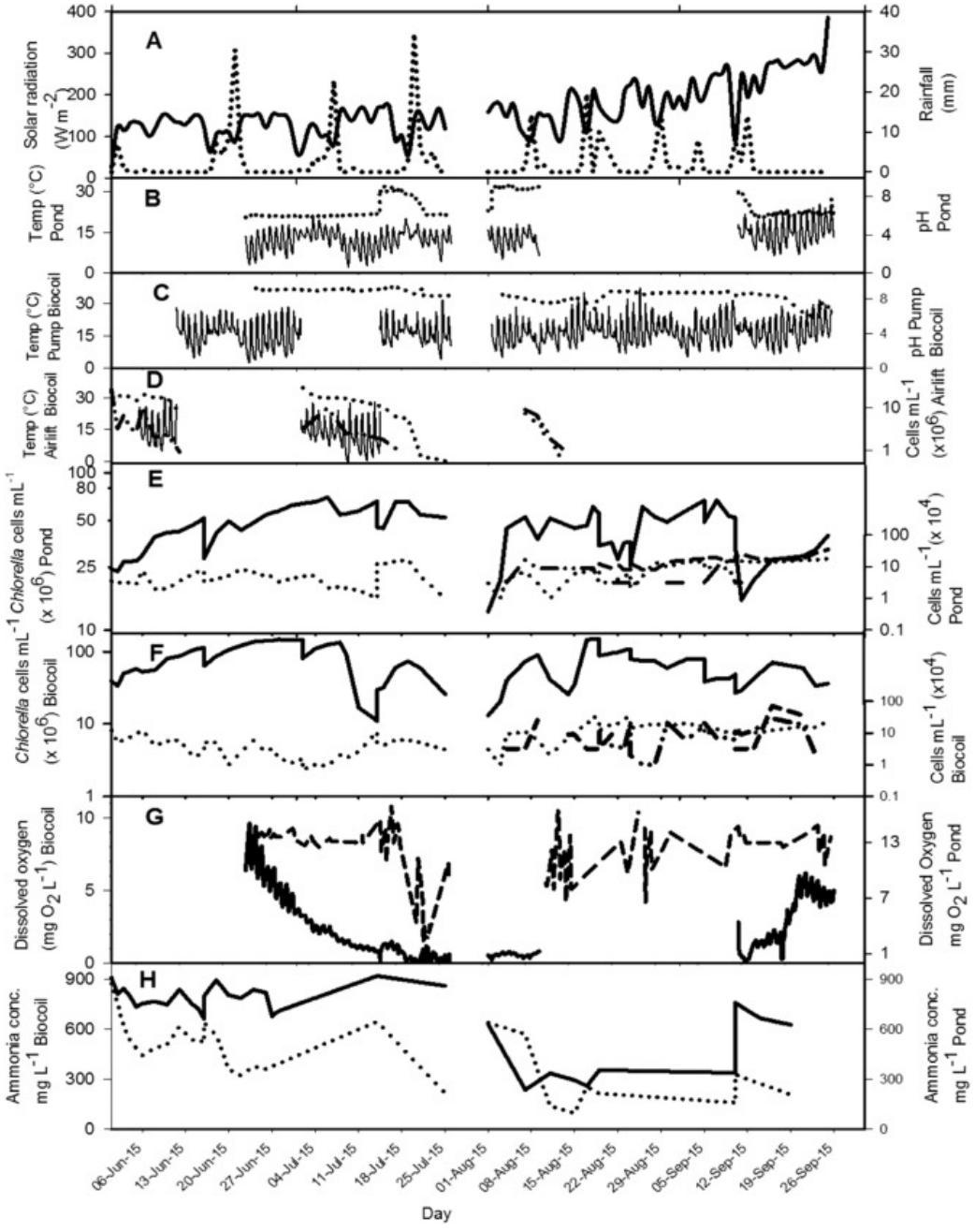

Fig. 2. Schematic diagrams, (***a***) airlift geometry II, (***b***) airlift geometry IV, (***c***) submersible pump-driven and (***d***) Biocoil configurations; and (***e***) frozen culture (arrows) on the inside of the degasser on 9 July 2015.

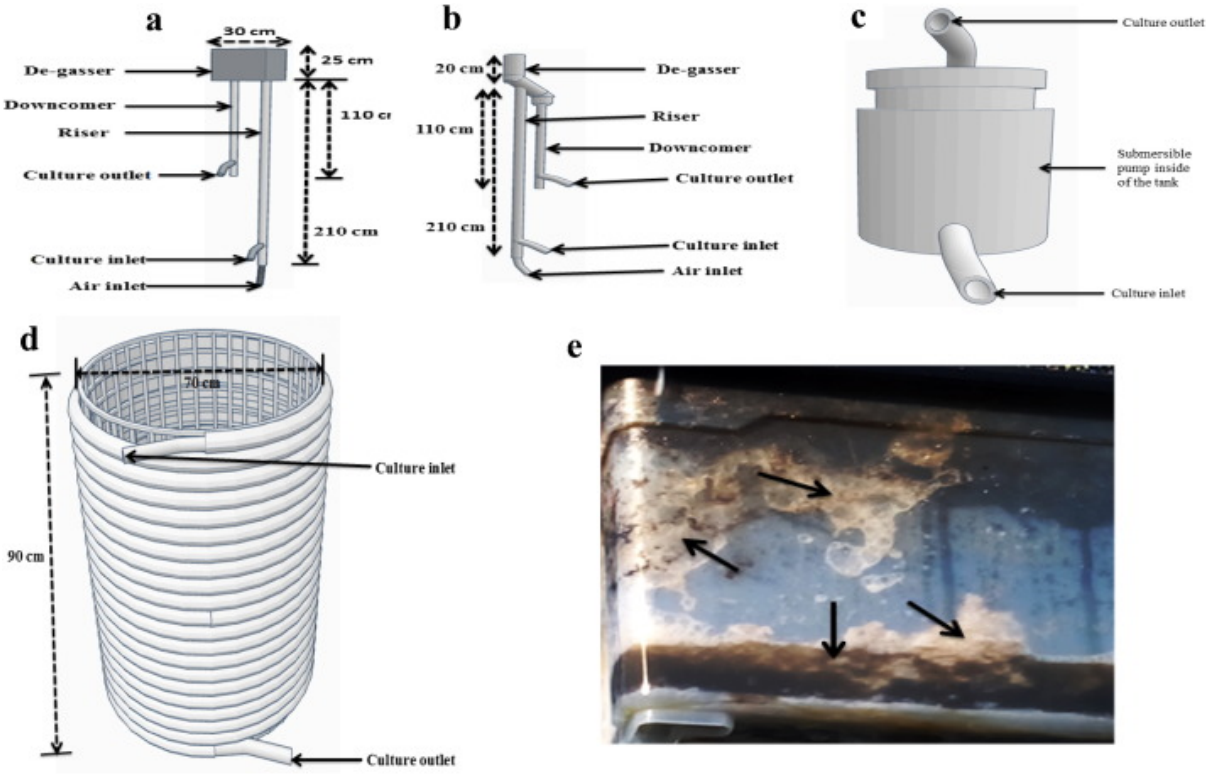

Table 1. Comparison of volumetric productivity, ammonium removal rate, biochemical, and chlorophyll compositions for the microalgae consortium grown in open raceway pond and the Biocoil treating sand-filtered ADPE.

Cultivation system	Month	Productivity ($mg\ L^{-1}d^{-1}$)	Ammonium removal rate ($mg\ NH_4\text{-}N\ L^{-1}d^{-1}$)	Biochemical content (%)			Chlorophyll content ($mg\ L^{-1}$)		
				Carbohydrates	Proteins	Lipids	*chl-a*	*chl-b*	$chlc_1 + c_2$
Biocoil	Jul	20 ± 0.35^{d}	15.5 ± 3.6^{a}	17.18 ± 1.5^{a}	25.14 ± 3.96^{b}	26.04 ± 11.67^{a}	3.67 ± 1.19	0.77 ± 0.41	0.85 ± 0.49
	Aug	47 ± 0.13^{f}	39.2 ± 9.5^{d}	39.20 ± 3.71^{bc}	31.38 ± 3.60^{d}	40.98 ± 7.65^{c}	6.15 ± 1.50^{c}	1.24 ± 0.34b	0.03 ± 0.05^{a}
	Sept	8.1 ± 0.23^{b}	14.7 ± 3.8^{a}	29.05 ± 5.2^{c}	21.49 ± 6.9^{ab}	33.34 ± 8.82^{b}	1.46 ± 0.98^{ab}	0.46 ± 0.06^{a}	0.07 ± 0.03^{a}
Raceway pond	Jul	8 ± 0.15^{c}	30.9 ± 10.1^{b}	23.72 ± 8.4^{b}	17.63 ± 2.0^{a}	26.24 ± 1.11^{a}	–	–	–
	Aug	24 ± 0.13^{e}	23.56 ± 5.7^{c}	34.96 ± 3.02^{bc}	25.97 ± 1.29^{b}	43.8 ± 2.08^{c}	1.76 ± 0.12^{b}	0.28 ± 0.06^{a}	0.03 ± 0.08^{a}
	Sept	3.1 ± 0.13^{a}	13.3 ± 8.8^{a}	30.88 ± 6.8^{c}	18.11 ± 5.4^{a}	26.35 ± 1.67^{a}	1.00 ± 0.05^{a}	0.13 ± 0.02^{a}	0.05 ± 0.03

Data are presented as means with standard errors, "-" Not determined. Along the column, the same letter denotes no significant differences (*t*-test, $P > 0.05$).