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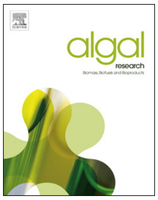

# Growth comparison of microalgae in tubular photobioreactor and open pond for treating anaerobic digestion piggery effluent

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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.

## 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

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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.