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Growth of microalgae on undiluted anaerobic digestate of piggery effluent with high ammonium concentrations

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ABSTRACT

Anaerobic digestate of piggery effluent (ADPE) is extremely high in ammonia toxic to many microorganisms. Bioprospecting and nutrient enrichment of several freshwater and wastewater samples combined and further acclimation resulted in a mixed culture containing at least three microalgae species capable of growing on undiluted ADPE. Outdoor growth of the mixed culture using raceway ponds showed potential for up to 63.7 ± 12.1 mg N-NH_4^+ L^{-1} d^{-1} ammonium removal from the ADPE. The microalgal consortium was dominated by *Chlorella* sp. and was stable at between 800 and 1600 mg N-NH_4^+ L^{-1}. Regulation of CO_2 addition to the ponds to maintain a pH of 8 increased chlorophyll content of the microalgal consortium. Average microalgal biomass productivity of 800 mg N-NH_4^+ L^{-1} culture conditions during five weeks semicontinuous growth was 18.5 mg ash-free dry weight L^{-1} d^{-1}. Doubling the ammonium concentration from 800 to 1600 mg N-NH_4^+ L^{-1} resulted in a 21% reduction of productivity, however the culture grown at 1600 mg N-NH_4^+ L^{-1} with the addition of CO_2 by keeping pH at pH = 8 led to a 17% increase in biomass productivity.

1. Introduction

A well-managed piggery should seek to handle and reuse wastewater appropriately, maintain control of odour emissions and aim to minimise its output of greenhouse emissions [1]. There is potential for improvement in the management and reuse of piggery wastewater. Piggery wastewater is very high in ammoniacal nitrogen and phosphorous as well as having significant chemical and biological oxygen demands [2]. These pollutants however can serve as beneficial nutrient sources for the growth of some microalgae. Microalgae produced in the context of pig production may provide income from the algal biomass produced as a source of animal or aquaculture feed [3], plant fertiliser [4] or biofuel [5].

Due to potential benefits of microalgae production incorporated into piggery systems, studies into the use of microalgae culture as a treatment for piggery wastewater have been ongoing for several decades [6–8]. So far however, results have failed to bring about widespread applications for the industry primarily due to concerns regarding the economic and environmental sustainability associated with pretreatment or dilution of the waste before growth of microalgae.

In the context of intensive pig production in places such as Australia, Europe and the USA, anaerobic digestion is a common treatment system [9–11]. Anaerobic digestion systems in Australian piggeries typically consist of covered outdoor ponds enabling the capture of biogas and output of partially treated water in the form of anaerobic digestate slurry. The adoption of this management approach for piggeries leads to the current scenario whereby anaerobic digestate of piggery effluent (ADPE) is now attractive as a microalgae growth medium. Current barriers to the adoption of microalgae culture for ADPE treatment include very high ammonia levels [12], high pH [13] and high turbidity (dark colour) [14]. The combination of high ammonia (around 1000 to 2000 mg N-NH_4^+ L^{-1}) and basic pH (above 8) in ADPE shifts the chemical equilibrium from NH_4^+ to NH_3 which is toxic to most organisms (microorganisms, aquatic ecosystems and terrestrial life such as vertebrates) [15–17]. NH_3 toxicity in microalgae has also been well documented, although the mechanisms for this are not well understood [13,18]. The effect of the toxicity at high pH is compounded as microalgae take up CO_2 during photosynthesis leading to a net increase in pH [19].

Although there are reports of raw piggery wastewater and ADPE use as microalgae growth medium, the majority of examples found in the current literature report the need for significant dilution for adequate microalgal growth [6,20-22]. The dilution of piggery wastewater for microalgae growth is not considered to be a viable option in most places with limitations of fresh water supply and

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potential problems with the disposal of this larger volume of water. Here we attempt to overcome some of these limitations by bioprospecting microalgae capable of growing well in undiluted ADPE.

We also examined the long term reliability of the growth of microalgae in diluted and undiluted ADPE. Testing reliability of any microalgae cultivation is necessary to indicate the potential practical application of the process [19]. Inorganic carbon is one of the main limits to the growth of microalgae [23]. The high pH of piggery anaerobic digestate results in lowering the amount of CO_2 available for microalgae growth. Therefore, we also tested cultivation of isolated microalgae with and without CO_2 addition to emulate growth as might be expected with the addition of a CO_2 source such as flue gas (as might be obtained through anaerobic digestion process and methane combustion). This CO_2 addition should also serve to improve the otherwise poor ratio of C:N which is likely to be another growth limiting factor [24]. Microalgae growth experiments were carried out in a series of four outdoor raceway ponds with a variety of synthetically increased ammonia concentrations to test the tolerance of the mixed microalgae consortium to high ammonia growth conditions as might be found in an ADPE based growth medium.

2. Materials and methods

2.1. Source and pre-treatment of ADPE

Samples of anaerobic digestate of piggery effluent (ADPE) from the Medina Research Station, Kwinana, Western Australia were collected and transported in 30 L plastic drums and used as the source of culture media for the duration of cultivation with no dilution. For the initial bioprospecting stage charcoal filtration [14] was used to remove some solids and reduce turbidity from the ADPE. For the remaining enrichment and cultivation experiments a slow feed sand filtration system was used to remove suspended solids and reduce the turbidity. A trickling speed of around 80 mL· min^{-1} was maintained through the filter which was made from a 30 L drum consisting of layered perforated PVC pipes, gravel and sand. Where required, ammonium chloride was added to the sand-filtered ADPE to allow for testing of higher ammonia concentrations. At the time of each ammonium chloride addition, pH was adjusted to pH = 9 using potassium hydroxide. Ammonia, total phosphorous, non-purgeable dissolved organic carbon (NPDOC) and non-purgeable total organic carbon (NPTOC) of ADPE were measured by the Marine and Freshwater Research Laboratory at Murdoch University. Methods used were: 2000 (Ammonia), 4700 (Total-P), 6000 (NPDOC) and 6000 (NPTOC) of 'Standard Methods for the Examination of Water and Wastewater' [25]. The effluent had an ammonium content of 240–690 mg $N\text{-}NH_4^+$ L^{-1}, total phosphorous of 33–43 mg P L^{-1}, NPDOC of 69–97 mg C· L^{-1}, NPTOC of 97–220 mg C· L^{-1}.

2.2. Bioprospecting and enrichment

Bioprospecting and enrichment for isolating suitable microalgae included sampling from water sources such as outdoor raceway ponds, an animal drinking trough in a university paddock, a waste processing facility and a secondary evaporation pond from a research piggery. Microalgae samples were then gradually scaled up in volume and concurrently the strength of ADPE and concentration of ammonia was increased in a stepwise manner to select for strains suitable for growth on undiluted ADPE. The enrichment cultures were carried out in aerated 2 L aquaria using approximately 200 mL of the freshwater source and made up to 1 L volume using charcoal filtered ADPE. Initial cultures were conducted indoors using fluorescent light at 440 μ mol photons m^{-2} s^{-1} in a controlled temperature room at 25 ± 3 °C with a 12:12 day:night cycle. Cultures were also grown outdoors using natural light between 215 and 700 μmol photons m^{-2} s^{-1} during day time with variable temperatures ranging from 2 °C overnight up to a maximum of 47 °C during the day.

2.3. Outdoor trials for examining microalgae culture reliability

Larger scale outdoor cultivations were also conducted to determine reliability of the microalgae cultures outdoors as well as the effects of different ammonium concentrations and CO_2 addition. Cultivation at a large scale [by] increasing the depth also provides benefits of greater aerial productivity as well as maximising nutrient removal rates.

Using a mixed microalgae culture obtained during bioprospecting screening and culture enrichment, outdoor cultivation was carried out using 1 m^2 fibreglass paddle-wheel driven raceway ponds during the winter months of 2013 at Murdoch University [26]. In order to provide a smooth transition to large scale cultivation the culture depth was gradually increased rather than immediately brought to it's maximum final capacity. The combination of initially using charcoal filtered ADPE before transitioning to sand-filtered ADPE along with the gradual increase in depth was expected to reduce the risk of culture collapse and allow for further adaptation and acclimation to outdoor culture conditions and much larger volumes. Once the depth and volume of the culture were operating at full capacity, the ammonium concentration was increased gradually to also provide opportunity for adaptation and selection of the most fit algal strains present in the mixed culture.

To this end, a volume of the combined bioprospecting cultures grown at the lab totalling 3.5 L was introduced to an empty raceway pond with around 15 L of charcoal filtered ADPE (pond 'g' in Fig. 1). The following day a further volume of charcoal filtered ADPE was added to the pond to make up a total of 50 L pond medium (around 5 cm depth). Over the course of two months the pond volume was gradually increased to 150 L total pond volume (15 cm depth) by the addition of fresh sand-filtered ADPE, and also a second pond (pond 'e' in Fig. 1) was established from the same culture. Fresh tap-water was also used to top-up any losses due to evaporation. From this point on, pond depth was maintained at 15–18 cm with variation due to rainfall and evaporation. The flow rate in the ponds was 20 cm s^{-1}.

From 5 June to 9 July two more ponds were added to the experiment (ponds 'd' and 'f' in Fig. 1) making a total of four ponds in use for cultivation. As the ammonium concentration had dropped during the previous cultivation period it was necessary to gradually increase the ammonium concentration to 800 mg $N\text{-}NH_4^+$ L^{-1} by the addition of ammonium chloride on average around every three days (see Section 2.1). At this time the other pond (pond 'd' in Fig. 1) was a control culture with no addition of ammonium. From 10th of July to 18th of August 2013 four 1 m^2 raceway ponds were operated in batch growth mode. In two of the ponds ammonium concentrations were maintained at 800 mg $N\text{-}NH_4^+$ L^{-1} (ponds 'd' and 'e' in Fig. 1). In the other two ponds ammonium concentrations were increased from 800 mg $N\text{-}NH_4^+$ L^{-1} to 1600 mg $N\text{-}NH_4^+$ L^{-1} stepwise over a 3 week period (ponds 'f' and 'g' in Fig. 1). One of the 800 mg $N\text{-}NH_4^+$ L^{-1} and one of the 1600 mg $N\text{-}NH_4^+$ L^{-1} ponds were supplemented with CO_2 using a pH-stat system set at pH = 8 [27] (ponds 'e' and 'g' in Fig. 1).

From 19 August to 25 September all ponds were operated in a semicontinuous culture mode with. Again one of the 800 mg $N\text{-}NH_4^+$ L^{-1} and one of the 1600 mg $N\text{-}NH_4^+$ L^{-1} ponds was supplemented with CO_2 using a pH-stat system set at pH = 8 continuing on from the previous experiment. Air temperature, irradiance, humidity and rainfall data were obtained from the Murdoch University weather station (http://wwwmet.murdoch.edu.au).