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# Nutritional profile and *in vitro* digestibility of microalgae grown in anaerobically digested piggery effluent

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ABSTRACT

Microalgal biomass grown in wastewater can be a sustainable source of animal feedstock. We have previously shown the feasibility of mass algal cultivation on undiluted anaerobic digested piggery effluent (ADPE). In this study, we evaluated the nutritional value, pathogen load, *in vitro* digestibility and potential physiological energy (PPE) of ADPE-grown microalgae as a potential feedstock for pigs. Pathogen load of ADPE-grown microalgae was within regulatory limits. Crude protein of ADPE-grown microalgae was higher than full fat soybeans but was much lower than conventional soybean meals (SBM) currently employed as a source of protein in pig feeds. The essential amino acid content of the microalgae was also lower than SBM. Fatty acid composition of the microalgae was favourable with an omega-3:omega 6 ratio of ~1.9, which may offer potential for value-adding use in some diets. *In vitro* digestibilities were higher in faeces than at the ileum and were lower for the defatted microalgal biomass. The (theoretical) net energy values of ground and bead-milled algae samples were found to be comparable to that of deshelled sunflower meal used as a feeding ingredient for pigs, but were lower than SBM.

## 1. Introduction

The surge in world population coupled with an increase in the average household income is projected to double the requirement of animal-based products (*e.g.* meat, milk and eggs) and challenge the biocapacity (*e.g.* forestry, fishery and crop reserves) of our planet [1]. The apparent increase in meat demand will most certainly overextend current livestock agricultural practices for conventional food crops such as corn and soybean, commonly used for the nourishment of food producing animals [2]. In addition, the consumption of corn and soybean as human food and their current exploitation as bioenergy feedstock poses a direct conflict to global nutrition security [2]. Thus, there is great need for alternative raw materials for animal feed production that are not only economical but also environmentally tenable.

Microalgal biomass is a potential candidate for the production of various commodities such as animal feed [3]. Microalgae have a significantly higher biomass productivity than any other photosynthetic organisms and most importantly, microalgal cultivation does not compete with food crops over arable land [4]. Microalgal biomass can impact animal growth and development by supplying a range of nutrients such as vitamins, minerals and essential fatty acids, affecting immune responses and fertility as well as improving animals' external appearances through skin pigmentation [4]. It is estimated that approximately 30% of total microalgal biomass cultivated around the world is currently sold as animal feed [5].

Nonetheless, various challenges and obstacles remain in realizing the true potential of microalgae biomass as a source of animal feed. Among the prominent factors limiting the commercialization of any algal production system is the overall economics [6]. Elevated cost factors such as the capital (Capex) and operating expenses (Opex) have significantly hampered the scaling up of these facilities especially for the production of low cost commodities such as bioenergy and animal feed [4]. Thus, a first priority should be focussed on optimizing production efficiency while successfully minimizing energy use and associated costs to achieve feasible yields of microalgae. Fertilisers are a major Opex for any algal production and the use of wastewater is an ideal solution for reducing such cost [7]. Anaerobic digestate piggery effluent (ADPE) is a wastewater that has to be treated before being released to the environment [8]. The cultivation of microalgae on ADPE would serve as an innovative strategy for animal waste management and the production of low-cost algal-based animal feed. Such an integrated system would most certainly allow for the following benefits

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1) Most piggeries primarily treat their manure effluent through anaerobic digestion for the production of methane.
2) The ADPE produced is rich in fertilisers that cannot be directly released into environmental water bodies.
3) Cultivation of microalgae on ADPE would allow for the assimilation of inorganic nutrients by algal cells to be converted into valuable components such as lipids, protein and carbohydrate.
4) The consumption of nutrients by algal cells would allow for the bioremediation of the ADPE.
5) Algal biomass produced from ADPE could be used a high nutritious feed source for animals such as pigs.

Previously, we have isolated a microalgal consortium capable of growing on undiluted ADPE with up to 1600 mg $L^{-1}$ ammonium $NH_4^+$ [10]. This selected microalgal consortium can also efficiently and reliably strip nutrients (*e.g.* over 40 mg $NH_4^+$-N $L^{-1}$ $d^{-1}$) from ADPE when using paddle-wheel driven raceway ponds and closed photobioreactors [10,11].

In the current study, we aim to evaluate the potential use of ADPE-grown microalgal biomass as a feed ingredient for pigs by 1) examining the nutritional and biochemical properties of ADPE-grown and harvested biomass as an alternative for soybean meal (SBM), 2) testing the bacterial load of biomass; and (3) evaluating *in vitro* digestibility of this biomass as a potential feed ingredient for pigs. Such a study would prove to be vital in the total risk analysis and feasibility evaluation of the use of ADPE-grown microalgae a feedstock for pigs.

## 2. Material and methods

### 2.1. Microalgae consortium and cultivation conditions

The microalgal consortium (*Chlorella* sp. and *Scenedesmus* sp.) used in this study has been described in our previous studies [10,11]. Anaerobically digested piggery effluent (ADPE) was obtained from the Medina Research Station (MRS) located in Kwinana, Western Australia (32.2376° S, 115.8285° E). Medina Research Station employs a covered biological anaerobic digestion pond to treat its wastewater [10]. Despite the anaerobic treatment process, the ADPE is still enriched with a very high inorganic nutrient load (*e.g.* nitrogen and phosphorous) at the point of discharge to the evaporation pond. The ADPE collected was sand-filtered and used for the cultivation of microalgae without any further pre-treatment [10]. Physiochemical properties of the sand-filtered ADPE were characterized using a Hanna Instruments COD and Multiparameter Photometer (HI 83099) based on the protocols and reagents provided by the manufacturer and are summarized in Table 1.

An 11 $m^2$ open raceway pond with a single paddle wheel (4 blades = approximately 30 cm.$s^{-1}$ mixing velocity) operated at a depth of 15 cm was employed for microalgal cultivation using ADPE. Samples were collected for determination of nitrogen concentration (N-$NH_3$ and N-$NO_3^-$) and COD at 11 am every second day during the batch and semicontinuous culture to calculate nutrient removal rates. Batch cultures represent the growth cycle of algal cells in media from an initial concentration till their highest cell density without any inflow or outflow of media or cultures. On the other hand, during semi-continuous cultivation, cultures were periodically harvested (50%) and replaced with fresh media whenever they reached maximum concentrations in order to maintain cells in exponential phase.

**Table 1**
Chemical composition of untreated and undiluted ADPE used for the growth of the microalgae (from Ayre et al. [10]).

| Parameter | Value |
|---|---|
| Ammonia (mg $L^{-1}$ $NH_4^+$-N) | 960–1000 |
| Total Phosphate, (mg $L^{-1}$ $PO_4$-P) | 25.0–26.5 |
| Nitrite (μg $L^{-1}$ $NO_2$-N) | 8.0–8.5 |
| Magnesium (Mg $L^{-1}$ mg) | 165–175 |
| Potassium (mg $L^{-1}$ K) | 530–545 |
| Total Iron (mg $L^{-1}$ Fe) | 8.5–9.5 |
| Nitrate (mg $L^{-1}$ $NO_3$-N) | 14.0 14.5 |
| Chemical Oxygen Demand, COD (mg $L^{-1}$) | 1200–1350 |
| Total nitrogen (mg $L^{-1}$ N) | 1050–1101 |

Samples for water nutrient analysis were centrifuged at 3000 rpm for 10 min. Supernatants were adequately diluted for analyses. Ammonia and chemical oxygen demand (COD) in cultures were also measured using a Hanna HI 83099 COD and Multiparameter Photometer. Microalgal biomass concentration as ash-free dry weight (AFDW) was measured in cultures during the growth period to calculate productivity rates [12].

### 2.2. Analytical methods

The algal biomass required for all analytical measurement in this study was harvested from the raceway pond using an industrial scale bucket centrifuge. The harvested algal biomass was subsequently dried at 60 °C (to prevent the degradation of cell composition) for a minimum of 12 h using a conventional oven. After drying, the dried biomass was ground using a domestic grinder and sieved down to 1 mm in size. For the purpose of *in vitro* digestibility analyses, a portion of the ground and sieved biomass was subjected to further pre-treatments such as bead milling and defatting. Milling was performed using a planetary ball mill (Across International PQ-N2). Agate jars and balls were used for milling the samples. The following combination of ball sizes were used: 4 pieces of the 20 mm size, 200 pieces of the 10 mm size, and 500 pieces of the 6 mm size (around 510 g of the balls per 100 g of dried and sieved algal biomass). This protocol used followed the manufacturer's recommendation of maintaining a ratio close to 1:5 of sample to grinding balls. The milling was performed at several intervals arriving at a total of one hour milling time. This consisted of four repeats of 15 min milling: 7.5 min rotating clockwise, followed by a one minute pause; then 7.5 min anticlockwise followed by a one-minute pause. Approximately 3 g of processed sample were collected after each 15-minute interval for chlorophyll *a* measurement as a method to determine the effectiveness of the grinding on the rupture of the algal cell walls (data not shown).

The defatting of the algal biomass was conducted by mixing 500 g of milled sample with 2 l of hexane [13]. The mixture was continuously mixed using a magnetic stirrer for 6 h. After mixing, the defatted biomass was subsequently oven dried at 60 °C to remove any remaining residue hexane and to deactivate trypsin inhibitors and lectins similar to the toasting step of soybean meals (SBM) [14]. The total lipid content of the initial and final samples were evaluated based on the methods of Bligh and Dyer (procedures described in Moheimani et al. [12]).

The algal biomass nutrient profile (*e.g.* protein, lipid, carbohydrates, vitamin and minerals) was analysed by Upscience Laboratories (Formerly InVivo Labs), Vietnam (www.upscience-labs.com), which is accredited as a reference laboratory for feed and pet food testing. All measurements were based on their standard methods and are summarized in supplementary data 1. The pathogenic bacteria content of the dried algal biomass was evaluated by the Food Hygiene Laboratory, Path West Laboratory Medicine, Western Australia (see supplementary data 2) (www.pathwest.health.wa.gov.au).

The *in vitro* digestibility of the ground, bead milled and defatted ADPE-grown microalgae biomass was tested by EuroFins Steins Laboratorium A/S (www.eurofins.dk). In brief, EFOS Pig (%) (in English; Enzymatic digestion of organic matter (OM)) determines the content of *in vitro* digestible organic matter for pigs. The sample was incubated with pepsin, followed by pancreatin and viscozyme, and the undissolved sample material was filtered off, dried and ashed. The solubility of OM is calculated by comparing the dry matter and ashes content after the enzyme treatment with dry matter and ash in the