Project Number & Title: 4A-106 Growth, development and use of algae grown on untreated and undiluted anaerobic digestion piggery effluent

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Aims and Objectives:

The main aims of this project were to test:
1. Sustainable cultivation of microalgae on undiluted and non-treated anaerobic digestion piggery effluent.
2. Microalgae growth optimisation as well as nutrient and CO_2 removal.
3. Nutrient composition of produced biomass and test the suitability of grown algae as a protein feed for pigs.
5. Co-anaerobic digestion of produced biomass with piggeries effluent.
6. Preliminary techno-economics.

Key Findings

Waste slurry produced from the primary treatment of anaerobic digestion of raw piggery effluent (ADPE) employed in most piggeries is still limited by elevated concentrations of ammonium that can be toxic to most living organisms.

The integration of microalgae cultivation as a subsequent step to anaerobic digestion has been proposed as a potential component of the Australian wastewater management strategy for piggeries to treat ADPE efficiently. In accordance, this project evaluated the growth, development and use of microalgal grown on undiluted and sand filtered ADPE. More specifically, in this work, we studied the long-term cultivation and growth of an isolated microalgal consortium consisting mainly of *Chlorella* sp. and *Scenedesmus* sp. that were capable on growing in undiluted ADPE while simultaneously optimizing limiting factors to their growth and productivity. Outdoor growth of the mixed algal culture on ADPE using raceway ponds showed potential for up to 63.7 ± 12.1 mg N-NH_4^+ L^{-1} d^{-1} ammonium removal from ADPE. The microalgal consortium was dominated by *Chlorella* sp. and was stable when grown between 800 and 1600 mg N-NH_4^+ L^{-1} ADPE. Average microalgal biomass productivity at 800 mg N-NH_4^+ L^{-1} ADPE during five weeks of 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 when cultures were grown at 1600 mg N-NH_4^+ L^{-1} with the addition of CO_2 at pH = 8 led to a 17% increase in biomass productivity.

Comparison between different algal cultivation systems (i.e. open ponds and closed photobioreactors) and different mixing mechanisms (paddle wheel and jets) were also evaluated to optimize the microalgal growth and increase nutrient removal rate. 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. Overall, similar average ammonium nitrogen removal rates in the submersible pump 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.

We also compared the turbulent mixing and nutrient removal efficiency of conventional paddlewheel driven raceway ponds (PWP) with customized jet nozzle mixed raceway pond (JNP) on microalgae grown in ADPE. Overall, the concentration of microalgae consisting mainly of Cyanobacteria and *Chlorella* sp. trended higher in the JNP over the PWP with the visible absence of diatoms in JNP. The ammonium removal rates (%) were also found to be significantly higher in the JNP (36.8 ± 3.93) than the PWP (23.5 ± 4.42).

Also as part of this study, the quality and suitability of grown biomass as a potential feedstock to pigs were assessed. We evaluated the nutritional value, pathogen load, *in vitro* digestibility and potential physiological energy (PPE) of ADPE-grown microalgae as a potential pig feedstock. Pathogen load of ADPE-grown microalgae was within regulatory limits. Crude protein and essential amino acid content was comparable with a number of other vegetable protein sources for pig, but was slightly lower in some essential amino acids than soybean meal (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 uses in some diets. *In vitro* digestibility, the digestibilities were higher in faeces than at the ileum and were lower for the defatted microalgae biomass. The (theoretical) net energy values of ground and bead-milled algae samples were found to be comparable to conventional de-hulled sunflower meal used as a feeding ingredient for pigs but were lower than SBM.

The feasibility of co-digesting manure and algae was also evaluated. In particular, we identified pre-treatment requirements for the algae, potential toxicity effect or inhibition of the microalgae grown on ADPE and optimal manure to algal loading ratio involved in the co-digestion process. The results suggested that the unbroken and broken algal sample with lipid removal treatments used did not significantly affect methane production, but broken cells yielded the lowest methane production. This suggests that it would be preferable to use algae unaltered.

The suitability of the ADPE- grown algal biomass as a fertilizer for crops and raw material for anaerobic digestion was also studied. A pot experiment was carried out with the following treatments: microalgae biomass and NH_4NO_3 at five N equivalent levels for 6 weeks. Wheat (*Triticum aestivum* L.) was used as the host plant in a randomized block design in three replicates. Utilization of the two N sources significantly improved the dry-harvest for wheat plants at the greatest application level compared with the control. The N sources and levels significantly affected the N uptake in shoots. The application of microalgae biomass revealed significant lower N in shoots compared to the NH_4NO_3. Results of this study indicated that microalgae biomass is an available nitrogen source for plants.

Finally, the cost assessment of integrating microalgae cultivation in existing piggeries to treat ADPE was conducted on multiple plausible scenarios to identify the economic viability of the proposed process. We conducted a preliminary techno-economics of microalgae cultures grown in paddle wheel driven raceway ponds and tubular photobioreactors for the treatment of undiluted ADPE at a medium (400 sows) and a large (2,000 sows) sized piggeries. The results indicated that the lowest production cost was achieved when productivity was highest. The most economical outcome was achieved for a large sized piggery using paddle wheel driven raceway ponds.

Application to Industry

Our findings highlighted the potential use and promise of the isolated *Chlorella* and *Scenedesmus* consortium for the bioremediation of ADPE and biomass production. To the best of our knowledge, this is the first study evaluating the potential of using microalgae to treat undiluted ADPE. Based on the outcome of the current study, raceway ponds can be recommended to the industry. While there is a need for further optimisation at pilot level, successful microalgae growth on ADPE indicates the potential of using these organisms for not only treating ADPE but also as a potential source of animal feed, fertiliser or bioenergy (methane) production. The generated biomass can also be sold as aquaculture feed. We highly recommend the Pork Industry to adopt the outcome of this study. However, there is a need for further onsite pilot and demonstration before commercialisation.