THE ANAEROBIC DIGESTION OF HALOPHYTIC MICROALGAE

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THE UNIVERSITY of ADELAIDE

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Declaration for a thesis that contains publications

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Thesis by Publication

This doctoral thesis is submitted as a portfolio of peer-reviewed publications according to the “PhD Rules & Specifications for thesis” of the University of Adelaide. The journals in which these papers were published or accepted are closely related to the research field of this work. The citation index is listed and the journals ranked in order of impact factor in reference to their scientific significance.

Journal Title	Impact Factor
Bioresource Technology	5.039
Journal of Algal Research	4.095
Journal of Water Process Engineering	N/A

The thesis is composed of the following:

Book chapter

1- Ward, A.J. Biofuel Bio-refinery Technologies, Vol. 2, Navid R. Moheimani et al: BIOMASS AND BIOFUELS FROM MICROALGAE chapter 16 The Anaerobic Digestion of Microalgae Feedstock, “Life-Cycle Environmental Impacts of Biofuels and Co-products” DOI 10.1007/978-3-319-16640_16

Peer reviewed scientific publications

1- Ward, A.J. Lewis, D.M. Green, F.B., (2014). Anaerobic digestion of algal biomass: A Review. Journal of Algal Research. 5:204-214
2- Nguyen, L.T.A. Ward, A.J. Lewis D., (2014). Utilisation of turbidity as an indicator for biochemical and chemical oxygen demand. Journal of Water Process Engineering. 4:137-142
3- Ward, A.J. Lewis, D. Ball, A., (2014). Halophytic microalgae as a feedstock for anaerobic digestion. Journal of Algal Research. 7:16-23
4- Ward, A.J. Lewis, D.M., (2015). Pre-treatment of halophytic microalgae and associated methane production. Bioresource Technology. 177:410-413
5- Erkelens, M. Ward, A.J. Lewis, D.M., (2014). Microalgae digestate effluent as a growth media for *Tetraselmis sp*. in the production of biofuels. Bioresource Technology. 167:81-86

Conference presentations that resulted from my PhD research

1- Ward, A.J. Lewis, D., (2014). The anaerobic digestion of microalgae biomass. (Presented at the Bioenergy Australia Conference, Sydney, Australia, 2014)
2- Ward, A.J. Lewis, D. Ball A., (2014). The anaerobic digestion of halophytic microalgae. (Presented at the International Society for Applied Phycology Conference, Sydney, Australia, 2014)
3- Ward, A.J. Lewis, D. Ball, A. (2013). The anaerobic digestion of saline biomass under high salinity conditions. (Presented at the Bioenergy Australia Conference, Sydney, Australia, 2012)

4- Ward, A.J. Lewis D.M., (2013). Anaerobic Digestion of marine microalgae biomass. (Presented at the International Marine Biotechnology Conference, Brisbane, Australia, 2013) (Received runner up best student presentation)

5- Ward, A.J. Ball, A. Harris P. Lewis, D., (2012). Methane production from saline microalgae utilising high salinity tolerant methanogen *Archaeal* microbes. (Presented at the Bioenergy Australia Conference, Melbourne, Australia, 2012)

6- Ward, A.J., Lewis, D.M. Ball A.S., (2012). Molecular comparison of Methanogenic *Archaeal* bacteria using nested and non-nested (PCR) amplicons. (Presented at the Asia Pacific Conference on Algae Biotechnology, Adelaide, Australia, 2012)

7- Ward, A.J. Lewis, D. Barker, G., (2011). The Influence of nutrient loads on the production rate of *Botryococcus braunii* in digested piggery effluent medium. (Presented at the International Society for Applied Phycology Conference, Halifax, Canada, 2011)

8- Ward, A.J. Lewis, D.M., (2010). Nutrient recovery and methane production from saline micro-algal biomass, (Presented at the Bioenergy Australia Conference, Sydney, Australia, 2010)

Poster Presentations

1- Ward, A.J. (2013). Molecular comparison of nested and non-nested PCR 16S rDNA polymerase chain reaction (PCR) amplicons. (Presented at the Bioenergy Australia conference, Sydney, Australia, 2013).

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ABSTRACT

The anaerobic digestion of microalgae is a potential environmentally feasible option for creating a renewable source of energy for industrial and domestic needs. Microalgae anaerobic digestion is a key unit process that integrates efficiency and beneficially into the production of microalgae derived biofuels. Anaerobic digestion culminating in methane fermentation improves the economic viability of microalgae liquid biofuel production and presents an opportunity for power generation from wastewater derived microalgae. However the anaerobic digestion of halophytic microalgae biomass is not straight forward due to several technical restraints including low concentration of digestible biodegradable substrate, recalcitrant substrate constituents, cell wall degradability and effects from salinity and associated metal ions.

To address the quantification of low biodegradable substrate associated with microalgae cultures, development of a high throughput methodology to determine the quantification of suspended microalgae biomass content and other water quality parameters via turbidity measurements was determined. The development of the new management tool allows faster operational control from a simple turbidity analysis, reducing time delays to fewer than 5 minutes and avoids expensive laboratory testing. Further development of this management tool will support the operational control for biofuel pond management and wastewater treatment plants. This management tool provides a rapid quantification of biomass and allows harvesting volumes to be calculated to allow consistent volatile solid and chemical oxygen demand loading to anaerobic digesters.

The anaerobic digestion of halophytic microalgae biomass however, has a significant challenge to be mitigated before this technology can be beneficial for the burgeoning microalgae industry. The halophytic microalgae biomass as a potential substrate feedstock for anaerobic digestion will have salinities > 35 ppt. To address this issue the first section of my PhD research focussed on the changes undertaken in the bacterial community associated with the anaerobic digestion of piggery effluent under increasing saline conditions with the aim of establishing a saline tolerant anaerobic digestion inoculum capable of digesting feedstock's under high salinity conditions.

Favourable results from this inoculum development study allowed the second part of the PhD research to be investigated where the anaerobic digestion of halophytic biomass was investigated utilising the inoculum established from the initial component of the reported study. Results of the later study demonstrated that a hyper saline inoculum was achieved and subsequent DGGE fingerprinting of the bacterial community detected several high salinity methanogens at a salinity of 7% and validated the establishment of a halo-tolerant anaerobic digestion community. Establishment of a halo tolerant anaerobic digestion community was further validated by significant methane production at the high 7% salinities. This inoculum was then used for all other reported studies.

Another major difficulty associated with the anaerobic digestion of microalgae is the need to disrupt the cell wall allowing the cell contents to be processed by the bacterial community. In this study I compared the methane production from lipid extracted, pre-treated disrupted and non-pretreated *Tetraselmis* sp. microalgae respectively. Results demonstrate that a methane production of 122 mL per g VS for the lipid extracted *Tetraselmis* sp. biomass. This result demonstrates that after the

extraction of lipid for use in biofuel production residual lipid extracted microalgae biomass is a viable feedstock for methane production. A methane production of 252 mL per g VS and 248 mL per g VS was reported for the non-disrupted algae and pre-treated disrupted *Tetraselmis* sp. respectively. This study also identified the ability of the anaerobic digestion microbial community to undertake cell lyses via microbial degradation of the *Tetraselmis* sp. microalgae. Cell lyses by the anaerobic digestion microbial community can offer a direct conversion pathway for energy production were whole biomass can be harvested and concentrated and directly fed to the anaerobic digester without energy intensive pre-treatment or processing being required.

Investigation was also undertaken to quantify the suitability of anaerobically digested halophytic *Tetraselmis* sp. microalgae digestate as a nutrient feed stock to form a closed loop nutrient system. To determine microalgae digestate suitability I established that the following factors needed to be observed: growth, lipid content, and the bacterial community diversity. Microalgae digestate was diluted according to the concentration of NH_4^+ content (20, 40, 60, 80 mg/L) and compared against a standard medium for *Tetraselmis* sp.. The growth rate on the microalgae digestate media was not as rapid as the F/2 standard medium and the high microalgae digestate media concentrations correlated with lower total lipid contents, additionally acyl carrier proteins (ACP) gene expression rates displayed lower lipid gene expression within high microalgae digestate treatments. Lastly, higher concentrations of microalgae digestate were correlated with a higher bacterial diversity in the bacterial community throughout the investigation. No significant difference in lipid production and satisfactory growth was recorded for the lower microalgae digestate treatments. These results confirmed the suitability of

microalgae digestate as a suitable nutrient source for use in the production of *Tetraselmis* sp. biomass for lipid and biofuel production.

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