

**STRAIN SELECTION AND OUTDOOR CULTIVATION OF
HALOPHILIC MICROALGAE WITH POTENTIAL FOR LARGE-
SCALE BIODIESEL PRODUCTION**

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DECLARATION

I declare that this thesis is my own account of my research and contains work which has not been previously submitted for a degree at any tertiary institution.

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ABSTRACT

The focus of this study was to identify microalgae isolates suitable for long-term outdoor cultivation at high salinities for biodiesel production. Screening and selection of locally collected halophilic microalgae isolates were based on the comparison of phenotypic traits such as growth rate, photosynthetic response and biomass productivity over a range of salinities, temperatures, light intensities and dissolved oxygen levels. Strain selection was also carried out in outdoor open raceway ponds.

The prasinophyte *Tetraselmis* and benthic diatoms were the most abundant of the microalgal species isolated. Indoor and outdoor comparative studies between 6 *Tetraselmis* strains- MUR 167, 219, 230, 231, 232 and 233- and the benthic diatom *Amphora coffeaeformis* MUR 158 showed that *Tetraselmis* was more suited for outdoor long-term cultivation in open raceway ponds. Disadvantages associated with *A. coffeaeformis* MUR 158 were a lower salinity tolerance (up to 5% NaCl only, as compared to 9% NaCl for *Tetraselmis*), slower growth and above all, the inability to outcompete *Tetraselmis* in outdoor open cultures.

Appreciable differences were observed between the 6 *Tetraselmis* strains, with *Tetraselmis* MUR 167, 230, 232 and 233 being the most promising strains for outdoor cultivation. Based on indoor determination of P_{max} values, I_k values, growth rates, lipid content and photosynthetic response over a range of salinities, temperatures, light intensities and dissolved oxygen, *Tetraselmis* MUR 233 was the dominant strain, followed by MUR 230, MUR 232 and MUR 167. Out of doors, MUR 167 was the most productive strain when cultivated in open raceway ponds. However, *Tetraselmis* MUR 230 and MUR 233 were characterised by more consistent and stable growth. Regardless of strain variation, growth and biomass productivity in outdoor ponds were significantly influenced by water quality and were highest at cell densities below 40×10^4 cells.mL⁻¹, salinities below 12% NaCl and at a temperature range of 20-25 °C.

The appropriate choice of solvent system, solvent to sample ratio and laboratory techniques for lipid extraction was also highlighted in this study. The modified Bligh and Dyer method was more efficient in extracting lipids from exponentially-grown cells as compared to the modified Folch *et al.* method, whereas no difference between both extraction methods was observed in cells from the stationary phase. Final lipid values were significantly influenced by methods employed for gravimetric measurements of lipid content.

In summary, this study showed that the halophilic *Tetraselmis* species is a suitable candidate for outdoor large-scale cultivation and that better performance and higher productivities in the open ponds can be achieved through careful strain selection and optimum culture management regime. This study also underlined the need for careful and critical assessment of reported lipid values in the literature since many factors influence lipid extraction and the final lipid content.

ACKNOWLEDGEMENTS

I clearly remember the time in my first week of candidature when I was grudgingly sorting through stacks of overgrown microalgae cultures and instead, eagerly anticipating the outdoor cultivation work which was much more exciting, or so I was told. Little was I prepared for what lay ahead and before I could realise, there I was, pushing barrels of seawater and salt bags around in the sweltering heat, bartering beer with workers from a food packaging company (Anchor Food Ltd.) for much-needed water drums, climbing in and out of water tanks to fix taps and leaks and desperately fighting the weather and pestering flies while tending to thick green cultures! What a journey! Through all this, I have come to really appreciate the art of microalgae cultivation and I am indebted to everyone who supported me in making this long-term research endeavour a success.

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