An assessment of inexpensive methods for recovery of microalgal biomass and oils

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in

Biotechnology

at Massey University, Palmerston North, New Zealand

Tawan Chatsungnoen

2015

ABSTRACT

Inexpensive processes for harvesting the microalgal biomass from the culture media and recovering oils from the harvested biomass are necessary for economically viable production of low-value products such as fuels. This study focused on harvesting of microalgae biomass from the culture broth by flocculation–sedimentation and recovery of oils from the harvested biomass using solvent-based extraction. Flocculation–sedimentation was explored for several marine and freshwater microalgae including *Choricystis minor* (freshwater), *Neochloris* sp. (freshwater), *Chlorella vulgaris* grown in freshwater; *C. vulgaris* grown in seawater; *Nannochloropsis salina* (seawater) and *Cylindrotheca fusiformis* (seawater), as a means for substantially concentrating the biomass prior to further dewatering by other methods. Aluminum sulfate and ferric chloride were investigated as cheap, highly effective, readily available in large quantities and innocuous flocculants. Flocculation–sedimentation behavior of the microalgae was evaluated with several flocculation conditions. The optimal microalgal biomass harvesting conditions identified in batch flocculation studies were applied to design and characterize a continuous flocculation–sedimentation system. The effect of the flocculant used and the water in the biomass paste on the extraction of oils were assessed in comparison with controls. The optimal solvent composition for extraction of the biomass paste was established. Using this solvent composition, the optimal extraction conditions (i.e. the volume of the solvent mixture relative to biomass, the extraction temperature and time) were identified using a 2^3 factorial experimental design.

Removal of more than 95% of the biomass from the broth by flocculation–sedimentation was shown to be possible for all the microalgae, but the required dosage of the flocculant depended on the following factors: the microalgal species; the ionic strength of the

suspending fluid; the initial concentration of the biomass in the suspension; and the nature of the flocculant. Irrespective of the algal species, the flocculant dosage was found to increase linearly with increasing concentration of the biomass in the culture broth. The flocculant dosage for a given level of biomass recovery under standardized processing conditions increased with an increase in the cell specific surface area in the range of 26–450 μm^2 $cell^{-1}$. Al^{3+} was a better flocculant than Fe^{3+} for some algae, but the situation was reversed for some others. The continuous flow biomass recovery was performed with *N. salina*, as this alga had the highest oil productivity among the species studied. With an aluminum sulfate dosage of 229 mg L^{-1} and a total flow rate of 22.6 mL min^{-1}, almost 86% of the *N. salina* biomass could be recovered from the broth within 148 min in the sedimentation tank. A prior flocculation–sedimentation treatment could greatly reduce the energy demand of subsequent dewatering by other methods. The flocculants adsorbed to the biomass were not removed by washing, but this did not hinder oil recovery from the biomass paste by solvent extraction. A modification of well-known Bligh and Dyer method could be used to recover more than 96% of the oils from *N. salina* biomass paste. The single-step modified extraction procedure was much superior to the Bligh and Dyer original. The optimal extraction conditions for *N. salina* biomass paste included a solvent mixture (chloroform, methanol and water in the volume ratio of 5.7:3:1) volume of 33 mL per g (dry basis) of the algae biomass; an extraction temperature of 25°C; and an extraction time of 2 h.

This work represents the first detailed study of the continuous flocculation–sedimentation process for harvesting *N. salina* biomass from the culture broth and the specific suitable solvent combination of chloroform, methanol and water for extracting algal crude oils from the *N. salina* biomass paste without a prior drying step.

ACKNOWLEDGEMENTS

I express my sincere gratitude to my principal supervisor Professor Yusuf Chisti for giving me a great opportunity to be part of his research team. I am thankful for his enthusiastic encouragement, patient guidance, precious advice, useful criticism, friendship and continuous support throughout this study. It was a pleasure to work under his supervision.

I would like to thank Associate Professor Pak Lam-Yu, my co-supervisor, for his constant encouragement and friendship. I am grateful to Dr Jonathan Godfrey, Institute of Fundamental Sciences, Massey University and his team for help and advice relating to statistical experimental design.

I am specially grateful for the financial support provided by Ministry of Science and Technology, Thailand, and a study leave provided by Maejo University, Thailand. I would like to thank the Minister Counsellor (Education) and her team at the Office of Education Affairs, Royal Thai Embassy, Australia, for looking after me during my study in New Zealand.

A very special thanks goes to Professor Emeritus Ian Warrington and Professor Songvut Phetpradap, a Massey University alumni and my former boss, for introducing me to Massey University, a perfect place to develop professional scientific knowledge and skills.

I would like to thank the staff of the School of Engineering and Advanced Technology Microsuite Lab, Anne-Marie Jackson, John Edwards, Julia Good and Nereda Corbett, for their assistance all through my study. They all were very kind and helpful. I would like to thank my colleagues, Ta, Ople, Sadia, Ziad, Azilah, Pat, Farhan and Natanam

for their valuable suggestions and exchanges of knowledge and skills. I enjoyed working with them and valued their friendship.

I would like to express my gratitude and deepest appreciation to my mother, my grandparents, my aunts, my uncles, my mother and father-in-law, my brothers and my sister-in-law for their love, great patience, understanding and support. My lovely wife, dear Paweena, without your support and encouragement, I could not have finished my study. I just would like to say thank you very much for everything you have done for me. I wish to express my love to daughter Manee and son Kawin for being so good.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES