RGC Ref. No.:
UGC/FDS25/E10/19
(please insert ref. above)

RESEARCH GRANTS COUNCIL
COMPETITIVE RESEARCH FUNDING SCHEMES FOR THE LOCAL SELF-FINANCING DEGREE SECTOR

FACULTY DEVELOPMENT SCHEME (FDS)

Completion Report
(for completed projects only)

Submission Deadlines:	*1. Auditor's report with unspent balance, if any: within **six** months of the approved project completion date.* *2. Completion report: within **12** months of the approved project completion date.*

Part A: The Project and Investigator(s)

1. Project Title

Development of an energy efficient-rapid microalgae cells harvesting and disruption method to facilitate the downstream processing of biofuel production by using a novel synthesized cationic polymer coated magnetic nanocomposites

2. Investigator(s) and Academic Department(s) / Unit(s) Involved

Research Team	Name / Post	Unit / Department / Institution
Principal Investigator	Chan Cho Yin / Lecturer I	FST/ THEi
Co-Investigator(s)	NA	NA
Others	Chan Kwan Shing / Research Assistant II (7 Oct 2020 to 31 Dec 2021)	FST/ THEi
Others	Leung Shu Kei / Research Assistant II (10 Jul 2022 to 31 Dec 2022)	FST/ THEi
Others	Leung Shu Kei / student helper supported by the item of Research Experience for Undergraduate Student during the period of 24 Jun 2021 to 22 Oct 2021)	FST/ THEi
Others	Lun Lai Ting / Final Year Project student (AY2020-2021)	FST/ THEi
Others	Leung Shu Kei / Final Year Project student (AY2021-2022)	FST/ THEi

3. Project Duration

	Original	Revised	Date of RGC / Institution Approval *(must be quoted)*
Project Start Date	1/1/2020	NA	NA
Project Completion Date	31/12/2021	30/6/2022	25/10/2021
Project Completion Date	30/6/2022	31/12/2022	16/6/2022
Duration *(in month)*	24	30	25/10/2021
Duration *(in month)*	30	36	16/6/2022
Deadline for Submission of Completion Report	31/12/2022	31/12/2023	16/6/2022

4.4 Please attach photo(s) of acknowledgement of RGC-funded facilities / equipment.

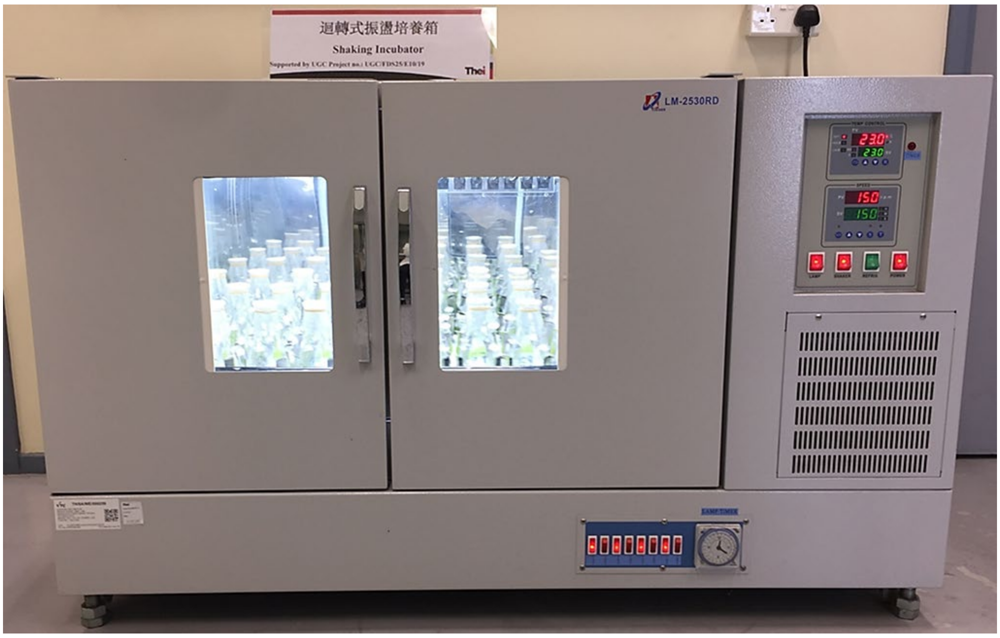

Figure 1. Shaking incubator with light-dark cycle used for the microalgae cultivation.

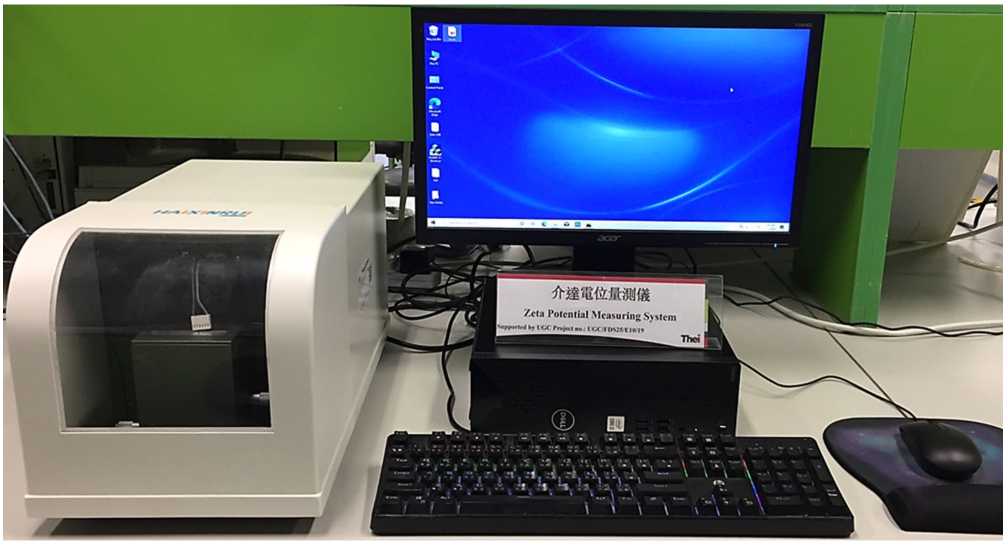

Figure 2. Zeta potential analyzer used for measuring the surface charge of magnetic nanocomposites.

Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. To synthesize a novel cationic-silica coated magnetic nanocomposites designed for effective microalgae cells collection by flocculation assisted with external magnetic field.

2. To develop an effective cells desorption method with significant enhancement of the lipid extraction efficiency by using a surfactant-based CTAB magnetic nanocomposites in order to shorten the cells disruption and lipid extraction downstream processes of the biofuel production.

3. To investigate the degree of reusability of cationic silica coated magnetic nanocomposites and spent culture medium for cells flocculation and cultivation of microalgae respectively.

4. To promote this new potential renewable energy to general public (students and industries) through experimental demonstration on how to achieve an energy efficient and rapid microalgae cells collection by using specific functionalized magnetic nanocomposites and deliver the research ideas and knowledge to classroom and final year project students.

5.2 Revised objectives

Date of approval from the RGC: NA

Reasons for the change: NA

5.3 Realisation of the objectives

(Maximum 1 page; please state how and to what extent the project objectives have been achieved; give reasons for under-achievements and outline attempts to overcome problems, if any)

Objective 1: To synthesize a novel cationic-silica coated magnetic nanocomposites designed for effective microalgae cells collection by flocculation assisted with external magnetic field.

Four different magnetic nanoparticles (i.e., bare-Fe_3O_4, NH_2-Fe_3O_4, citrate-80C-Fe_3O_4 and PDDA-coated Fe_3O_4) were synthesized and tested for the microalgae cell flocculation and magnetic separation processes. In the presence of 10‰ salinity which was a growth condition required for the selected marine green microalgae *Tetraselmis* sp., after gentle stirring of the microalgae culture with PDDA-Fe_3O_4 for 2 min and subsequent magnetic separation for 45 sec, results showed that 96.67% cell harvesting efficiency can be achieved. By comparing with other cell separation methods like sedimentation and auto-flocculation, an efficient and energy saving approach was successfully demonstrated for the microalgae cell separation by using the newly synthesized cationic PDDA-Fe_3O_4 nanocompsites.

Objective 2: To develop an effective cells desorption method with significant enhancement of the lipid extraction efficiency by using a surfactant-based CTAB magnetic nanocomposites in order to shorten the cells disruption and lipid extraction downstream processes of the biofuel production.

Since the current synthesized PDDA-coated Fe_3O_4 can achieve a significant higher cell harvesting efficiency than previously proposed surfactant-based CTAB magnetic nanocomposites (only ~50% cell harvesting efficiency shown) that a simple and effective cell desorption method by using mild condition such as pH adjustment was selected. Results showed that when the cells-PDDA-coated Fe_3O_4 mixture was adjusted to pH 4-5 by 0.5 M HCl, 93-80% of microalgae cells can be quickly recovered after 5 min of desorption, the collected cells were treated by alkaline reagent assisted with ultra-sonication, and then further reacted with organic solvent mixture for conducting cell disruption and lipid extraction respectively, in the downstream processes. While the collected PDDA-coated Fe_3O_4 can be easily reused for the next cycles.

Objective 3: To investigate the degree of reusability of cationic silica coated magnetic nanocomposites and spent culture medium for cells flocculation and cultivation of microalgae respectively.

The magnetic nanocomposites PDDA-iron oxide can be further reused for 3 cycles with 93.4-80.5% cell harvesting efficiency as observed. PDDA-iron oxide can be easily separated from the microalgae cells after 5 min gentle mixing at pH 4-5 by using 0.5 M HCl. On the other hand, after cell flocculation and magnetic separation, reuse of spent culture medium was conducted for 3 cultivation cycles without showing significant drop in the cell densities (~92-88% initial) although essential pretreatment on spent culture medium such as removal of dissolved organic metabolic wastes (released from microalgae cells) and nutrient replenishment were required.

Objective 4: To promote this new potential renewable energy to general public (students and industries) through experimental demonstration on how to achieve an energy efficient and rapid microalgae cells collection by using specific functionalized magnetic nanocomposites and deliver the research ideas and knowledge to classroom and final year project students.

The experimental setup like magnetic flocculation and separation was demonstrated to different high school and tertiary school students as well as related industrial sectors (i.e., R&D or consultancy companies) during campus and laboratory visits. Besides, the concepts of energy saving using microalgae in tertiary wastewater treatment and renewable energy such as biofuel production were delivered in different teaching courses such as Final Year Project (SEV5498/5499), Water and Wastewater Engineering (SEV4221), Biological Wastewater Engineering (SEV5342) and Industrial Wastewater Treatment (SEV5441).

5.4 Summary of objectives addressed to date

Objectives *(as per 5.1/5.2 above)*	**Addressed** *(please tick)*	**Percentage Achieved** *(please estimate)*
1. To synthesize a novel cationic-silica coated magnetic nanocomposites designed for effective microalgae cells collection by flocculation assisted with external magnetic field.	√	100%
2. To develop an effective cells desorption method with significant enhancement of the lipid extraction efficiency by using a surfactant-based CTAB magnetic nanocomposites in order to shorten the cells disruption and lipid extraction downstream processes of the biofuel production.	√	100%
3. To investigate the degree of reusability of cationic silica coated magnetic nanocomposites and spent culture medium for cells flocculation and cultivation of microalgae respectively.	√	100%
4. To promote this new potential renewable energy to general public (students and industries) through experimental demonstration on how to achieve an energy efficient and rapid microalgae cells collection by using specific functionalized magnetic nanocomposites and deliver the research ideas and knowledge to classroom and final year project students.	√	100%

6. Research Outcome

6.1 Major findings and research outcome
(Maximum 1 page; please make reference to Part C where necessary)

A rapid and energy-efficient biomass collection method for green microalgae *Tetraselmis* sp. was developed by using synthesized cationic magnetic nanocomposites. The reason of selecting *Tetraselmis* sp. because it has been reported with a high lipid content and fast growth rate (cell growth was monitored by optical density at 600 nm during 21 days of cultivation) that it can be potentially used for biofuel production. In addition, this species also can tolerate a wide range of salinities (i.e., 10‰ to 33‰ salinity of seawater), so a more flexible design could be used for their cultivation. Another selected brown marine microalgae *Isochrysis galbana* has been observed with lower cell densities and cannot be easily flocculated by the synthesized nanocomposites due to its smaller cell size, therefore, only *Tetraselmis* sp. was selected for further investigation. In this study, 4 different magnetic nanoparticles, i.e., bare Fe_3O_4, NH_2-Fe_3O_4, citrate-80C-Fe_3O_4 and polydiallyldimethylammonium (PDDA) coated iron oxide (PDDA-Fe_3O_4) were synthesized and their performances on cell flocculation and magnetic separation for *Tetraselmis* sp. were compared. Besides, for better understanding of the flocculation process, characterization study of different synthesized nanocomposites was conducted including transmission electron microscopy (TEM), vibrating sample magnetometer (VSM) and zeta potential, etc.

The synthesized magnetic nanoparticles were observed to have small particle size (<15 nm) and bore a high superparamagnetic property (86.3 to 73 emu/g) that such small nanoparticle size and high superparamagnetic nature of the synthesized nanocomposites resulted in rapid neutralization of the cell surface charge (-ve) and flocculation of the destabilized microalgae cells can be easily achieved. Results showed that when adding 8 mg PDDA coated iron oxide into 5 mL microalgae culture at initial pH 9 (~1:1 dried weight ratio between PDDA-coated Fe_3O_4 and cells), after gentle stirring for 2 min, 96.67% cell harvesting efficiency was observed. By comparing to other synthesized magnetic nanocomposites (i.e., 25 to 60% efficiency), such high cell harvesting efficiency observed in PDDA coated iron oxide was due to its higher positively charged density as shown in zeta potential analysis (+36.9 mV at pH 7.45). Besides, the flocculated cells-PDDA nanocomposites mixture can be easily collected after short period of magnetic separation (45 sec) by using a 200 mT permanent magnet. As a result, the overall processing time, treatment volume and energy requirement can be significantly reduced **(results shown in publication: *Water 2023, 15, 545*)**.

Effective cell desorption was achieved by simple pH adjustment and results showed that when the collected cells-PDDA-Fe_3O_4 mixture was adjusted from initial pH 9 to pH 4-5 by using 0.5 M HCl, after 5 min stirring, ~93-80% of cells can be recovered and subjected for further downstream process. The collected microalgae cells were treated by 0.5 M NaOH assisted with ultra-sonication, the degree of cell disruption can be estimated under light microscopy by using trypan blue staining method, while intact cells were not stained and remained in green. Results showed that using ultra-sonication in alkaline condition can enhance the cell disruption. Organic solvent mixture (chloroform-methanol 2:1 v/v) can be used for lipid extraction while further optimization of different parameters such as temperature and working pH will be required to increase the lipid extraction efficiency. On the other hand, the collected PDDA-Fe_3O_4 after magnetic separation and H_2O wash can be reused for at least 3 more flocculation cycles without showing significant drop in harvesting efficiency (93.4-80.5%).

Since ~25% growth inhibition was observed in a trial of direct reuse of spent culture medium (with nutrient replenishment) that pretreatment of spent culture medium by charcoal (0.2-0.3% w/v) was performed to reduce the content of dissolved organic matter (i.e., metabolic wastes of cells). After 15 min stirring, charcoal was filtered out and the pretreated spent medium was supplemented with the original nutrient level, results showed that 3 cultivation cycles can be further conducted without showing significant drop in cell densities (92-88% initial).

6.2 Potential for further development of the research and the proposed course of action *(Maximum half a page)*

The performance of synthesized magnetic nanocomposites PDDA-Fe_3O_4 used for cell harvesting should be further evaluated for different microalgal species before the application for large-scale biofuel production in the future. Since different cultivation conditions, such as salinity and medium pH may be required as well as different microalgae cell size may significantly affect the cell flocculation process, for example, the synthesized PDDA-Fe_3O_4 cannot perform very well at too high salinity (i.e., 33‰) that suppression of cell flocculation was observed (i.e., 50-55% cell harvesting efficiency) while such high salinity condition may be strictly required for certain marine microalgal species, thus it is necessary to overcome this suppression, for example, by increasing the dosage of magnetic flocculants used or prolonging the flocculation period. Besides, apart from energy and water saving concerns, techno-economic analysis should be conducted for this newly developed method to estimate the overall costs required (i.e., nanocomposites synthesis and operational costs) when comparing with different existing cell collection methods like centrifugation and filtration.

7. Layman's Summary

(Describe in layman's language the nature, significance and value of the research project, in no more than 200 words)

For achieving the goal of sustainable development, different sources of renewable energy should be explored to reduce the reliance on fossil fuel combustion, microalgae have been recently applied for biofuel production. However, 20-30% of overall energy consumption is used for the steps like microalgae cell collection and downstream processing that hinders its potential large scale applications, the energy consumption for cell collection should be significantly reduced by using new technological approach. In this study, an effective microalgae cell collection method is developed by using newly synthesized magnetic nanocomposites, which can be easily bound with the microalgae cells within short period of time and the cells-nanocomposites mixture can be rapidly collected by using an external magnetic field. Moreover, by adjusting the pH of reaction mixture, the microalgae cells can be separated from the nanocomposites, and the collected cells are further treated in the downstream processes for biofuel production. The magnetic nanocomposites can be reused for 3 cycles of cell collection. On the other hand, the spent culture medium can be reused for 3 cycles of cell cultivation although pretreatment is required to remove the impurities. Thus the overall energy and water consumption in this microalgae industry can be significantly reduced.

Part C: Research Output

8. Peer-Reviewed Journal Publication(s) Arising Directly From This Research Project

(Please attach a copy of the publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				**Author(s)** *(denote the corresponding author with an asterisk*)*	**Title and Journal / Book** *(with the volume, pages and other necessary publishing details specified)*	**Submitted to RGC** *(indicate the year ending of the relevant progress report)*	**Attached to this Report** *(Yes or No)*	**Acknowledged the Support of RGC** *(Yes or No)*	**Accessible from the Institutional Repository** *(Yes or No)*
Year of Publication	**Year of Acceptance** *(For paper accepted but not yet published)*	**Under Review**	**Under Preparation** *(optional)*						
2023				Kwan-Shing Chan, Shu-Kei Leung, Sammie Sum-Yi Wong, Shui-Shing Chan, Dawson Wai-Shun Suen, Chi-Wing Tsang, Cho-Yin Chan*	Development of an Energy-Efficient Rapid Microalgal Cell-Harvesting Method Using Synthesized Magnetic Nanocomposites *(Water 2023, 15(3), 545)*	No	Yes	Yes	To be updated

9. Recognized International Conference(s) In Which Paper(s) Related To This Research Project Was / Were Delivered

(Please attach a copy of each conference abstract)

Month / Year / Place	**Title**	**Conference Name**	**Submitted to RGC** *(indicate the year ending of the relevant progress report)*	**Attached to this Report** *(Yes or No)*	**Acknowledged the Support of RGC** *(Yes or No)*	**Accessible from the Institutional Repository** *(Yes or No)*
March 2023, Singapore	Effective Harvesting of Microalgae Biomass by using Novel Synthesized Cationic Polymer Coated Magnetic Nanocomposites	2023 4th Asia Conference on Renewable Energy and Environmental Engineering (AREEE 2023). Singapore. 24-26, March 2023 (online Oral presentation) (Best Conference Presentation Award).	No	Yes	Yes	To be updated

10. Whether Research Experience And New Knowledge Has Been Transferred / Has Contributed To Teaching And Learning

(Please elaborate)

- Selected experiments such as microalgae cultivation, investigation of microalgae cell growth parameter and magnetic separation of microalgae cells were arranged to provide scientific and practical training to 2 Final Year Project students (SEV5498/5499) and 2 junior research assistants.
- The research ideas and results obtained by using magnetic collection of microalgal cells for achieving energy saving in tertiary treatment of wastewater and biofuel production have been discussed in different modules such as SEV4221 Water and Wastewater Engineering, SEV5342 Biological Wastewater Engineering and SEV5441 Industrial Wastewater Treatment.
- An experiment setup has been demonstrated in laboratory to share the research ideas with students and visitors.

11. Student(s) Trained

(Please attach a copy of the title page of the thesis)

Name	Degree Registered for	Date of Registration	Date of Thesis Submission / Graduation
[redacted]	Degree of Bachelor of Engineering (Honours) in Environmental Engineering and Management	Sept 2016	Jun 2020 / Dec 2020 (thesis title page was submitted in the 1st progress report)
[redacted]	Degree of Bachelor of Engineering (Honours) in Environmental Engineering and Management	Sept 2018	May 2022 / Dec 2022 (thesis title page was submitted in the 2nd progress report)

12. Other Impact

(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, teaching enhancement, etc.)

Best conference presentation has been awarded in AREEE 2023. Moreover, PI has been invited to serve as technical committee in coming conference AREEE 2024.

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13. Statistics on Research Outputs

	Peer-reviewed Journal Publications	Conference Papers	Scholarly Books, Monographs and Chapters	Patents Awarded	Other Research Outputs (please specify)	
					Type	No.
No. of outputs arising directly from this research project	1	1	NA	NA		

14. Public Access Of Completion Report

(Please specify the information, if any, that cannot be provided for public access and give the reasons.)

Information that Cannot Be Provided for Public Access	Reasons
NA	NA