RGC Ref. No.:
UGC/FDS16/M09/20
(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

Chitosan-flocculated microalgal biomass as a supplement of food waste fish feed and its enhancement effects to growth and immunity of *Scortum barcoo*

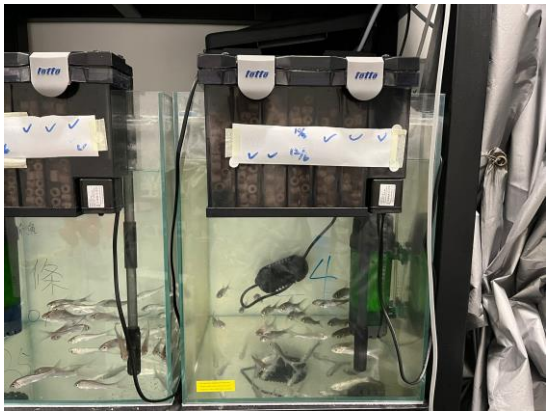
2. Investigator(s) and Academic Department(s) / Unit(s) Involved

Research Team	Name / Post	Unit / Department / Institution
Principal Investigator	Dr CHAN Sidney Man-ngai Assistant Professor	School of Science and Technology Hong Kong Metropolitan University
Co-Investigator	Dr SZE Eric Tung-po Adjunct Associate Professor	Department of Chemistry The Chinese University of Hong Kong
Co-Investigator	Dr MO Wing-yin Assistant Professor	School of Science and Technology Hong Kong Metropolitan University

3. Project Duration

	Original	Revised	Date of RGC / Institution Approval *(must be quoted)*
Project Start Date	01/01/2021	NA	
Project Completion Date	31/12/2022	30/06/2023	02/06/2022
Duration *(in month)*	24	30	02/06/2022
Deadline for Submission of Completion Report	31/12/2023	30/06/2024	02/06/2022

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



Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. *To select a robust and non-toxic microalgal species as supplement for fish feed production*
2. *To optimize the microalgal biomass production supported by the collected food waste leachate in an internal illumination photobioreactor for fish feed production*
3. *To optimize a cost-effective microalgal biomass harvesting technique, chitosan flocculation, for fish feed production*
4. *To evaluate the effects of microalgae- and chitosan-supplemented food-waste fish feed on the growth and nutritional value of Scortum barcoo*
5. *To elucidate the effects of microalgae- and chitosan-supplemented food-waste fish feed on the non-specific immunity of Scortum barcoo*

5.2 Revised objectives

Date of approval from the RGC: N/A

Reasons for the change: _____

_____

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 select a robust and non-toxic microalgal species as supplement for fish feed production

- Objective 1 was fully achieved.
- A total of nine different microalgal isolates were cultivated in different ratios of raw food waste leachate or food waste anaerobic digestate (AD) to identify a good candidate for biomass production. The optical density of the cultures, representing the growth of the isolates, were measured for 14 days and compared. *Chlorella* sp., a microalgal isolate from a local river, cultivated in AD (25, 50 75%) had optical densities even higher than that of the control (cultured in BG-11 culture medium) and was selected for the latter study.

Objective 2

To optimize the microalgal biomass production supported by the collected food waste leachate in an internal illumination photobioreactor for fish feed production

- Objective 2 was fully achieved.
- The setup for later on biomass production was established. Different setups of bioreactors with aeration were compared and a series of small size (2 L) bioreactors illuminated with external lighting was selected for lab scale production in this study. *Chlorella* sp. cultivated with different ratios of AD (1 to 40%) diluted with tap water in the selected bioreactor setup were compared. *Chlorella* sp. cultivated in 20% AD showed highest biomass productivity (171 mg L^{-1} day^{-1}) and lipid productivity (19.6 mg L^{-1} day^{-1}) were selected for latter study.

Objective 3

To optimize a cost-effective microalgal biomass harvesting technique, chitosan flocculation, for fish feed production

- Objective 3 was fully achieved.
- Different dosage of chitosan (0 to 100 mg chitosan L^{-1} culture) were mixed with microalgal cultures to form flocs for harvesting the biomass in the cultures. The microalgal biomass flocculation efficiency of different chitosan dosages were measured and compared. The optimal dosage was found to be 52.8 mg chitosan g^{-1} microalgae.

Objective 4

To evaluate the effects of microalgae- and chitosan-supplemented food-waste fish feed on the growth and nutritional value of *Scortum barcoo*

- Objective 4 was fully achieved.
- Due to the border control during the COVID pandemic, supply of fish was significantly affected. Another popular and available edible fish, *Hypophthalmichthys nobilis*, was used to perform the project. Fish feeds with 1 to 10% weight replaced by microalgal biomass were fed to fish for two months. The relative length gain, relative weight gain and feed conversion ratio of fish were monitored and compared. The performance of microalgae fish feeds were found to be similar to that of commercial feed.

Objective 5

To elucidate the effects of microalgae- and chitosan-supplemented food-waste fish feed on the non-specific immunity of *Scortum barcoo*

- Objective 5 was fully achieved.
- Fish feeds with 1 to 10% weight replaced by microalgal biomass were fed to fish for two months. Non-specific immunological responses, such as serum immunoglobulin, were

monitored and compared. Microalgae fish feeds did not show significant effect to the non-specific immunity of fish.

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 select a robust and non-toxic microalgal species as supplement for fish feed production	✓	100
2. To optimize the microalgal biomass production supported by the collected food waste leachate in an internal illumination photobioreactor for fish feed production	✓	100
3. To optimize a cost-effective microalgal biomass harvesting technique, chitosan flocculation, for fish feed production	✓	100
4. To evaluate the effects of microalgae- and chitosan-supplemented food-waste fish feed on the growth and nutritional value of *Scortum barcoo*	✓	100
5. To elucidate the effects of microalgae- and chitosan-supplemented food-waste fish feed on the non-specific immunity of *Scortum barcoo*	✓	100

6. Research Outcome

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

A total of two journal manuscripts, one patent application and three conference papers were generated to disseminate the research findings of this study.

In this study, a robust local microalgal isolate, *Chlorella* sp., was successfully screened out for fish feed supplement production. The isolate can be cultivated in a wide range of dilutions of filtered food waste anaerobic digestate (AD) with tap water (1, 5, 10, 20, 40% AD). Under the optimized conditions for fast growing established in this project (20% AD), the isolate showed productivity exceeding that of the control (BG-11 culture medium), with a biomass productivity of 171 mg microalgae L^{-1} day^{-1} and a lipid productivity of 19.6 mg fatty acid L^{-1} day^{-1} at the end of 7 days in a bioreactor setup. Depletion of dissolved nitrogen and dissolved phosphate were detected in all AD levels reflecting that the microalgae actually removing the pollutants in the diluted AD. Furthermore, it was found that the nutrient profile of microalgae cultivated with different dilutions of AD were different. Crude lipid content in the microalgal biomass decreased with increasing AD level (29.9 to 17.9% W/W). However, the fatty acid content increased from 1% AD, show a peak at 10% AD and dropped with further higher % AD.

Furthermore, the present study demonstrated a simple method for harvesting suspended microalgal biomass for fish feed production. Chitosan, a non-toxic flocculating agent derived from seafood waste, was applied to harvest the microalgal biomass suspended in diluted AD. For diluted AD and other medium with a salinity below 10‰, the optimal chitosan dosage for quick settling (4 minutes) was found to be 52.8 mg chitosan g^{-1} microalgae. By simple cloth filtering, the flocculated biomass can be collected easily, so that energy-intensive centrifugation processes can be avoided.

The above results were delineated in a manuscript for publication (Attachment 2).

More importantly, a fish feed formula consisted of fermented food waste, chitosan-flocculated *Chlorella* sp. was developed in this project (Table 6.1). The performance of this microalgae fish feed was comparable to that of commercial feed.

This fish feed formula and the method of production was delineated as a patent application (Attachment 6).

Table 6.1 The composition of microalgae fish feed developed in this project

Component	**Quantity ($g\ kg^{-1}$)**
Food Waste	400
Soybean Meal	350
Chitosan-Flocc'd Algae	100
Cornmeal	92
Baker's Yeast	50
Vitamin Mix	5
L-Choline	3

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

In this project, it has been demonstrated the possibility of integrating different waste-to-resources ideas into a product that has comparable performance with commercially available counterparts. The project shows the potential of using food waste, microalgae and chitosan for producing fish feed. Indeed, microalgae are known to be rich in nutrients including carbohydrate, protein, lipids, carotenoids, vitamins and minerals. However, different microalgal species has different nutrient profile and this profile can be significantly affected by the cultivation conditions. For future study, we should keep studying the characteristics of different microalgal species and identify their potentials for specific applications. By applying techniques of microalgae biorefinery, it is possible to purify nutrient materials from different microalgal biomasses. Fish feed and other dietary products can be prepared from multiple microalgae-derived materials instead of applying a single microalgal biomass directly. This allows much higher flexibility for modifying the fish feed formula to cater specific needs for different species or different growth stages.

7. Layman's Summary

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

A fish feed production using an integrated waste-to-resource approach has been successfully demonstrated in this project. Food waste, a type of waste that is difficult to get rid of, was fully utilized to produce an environmental-friendly fish feed for edible fish. The solid fraction of food waste was used as the base material for fish feed production after fermentation. The biomass of a microalga *Chlorella* sp. was produced using diluted anaerobic digestate, a polluting liquid generated during the treatment of food waste, and used as a dietary supplement fortified to the fish feed. Chitosan, the reagent used for the harvesting of microalgal biomass, was also a product derived from seafood waste. The overall growth and health of bighead carp, a popular edible fish, feeding on a fish feed with 10% microalgae was comparable to that of commercial feed revealing the potential of applying microalgae in a sustainable production of fish feed.

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)*						
2022				Yu YM, Poon PMY, Ashok SA, **Chan SMN**, Lee FWF, Mo WY, Sze ETP*	Colonization of *Lactobacillus rhamnosus* GG in *Cirrhinus molitorella* (mud carp) fingerling: evidence for improving disease resistance and growth performance. Applied Microbiology, 2022, 2(1), 175-184	Yes 2021	Yes [Attachment 1]	Yes	Yes
			Yes	Tam TSC, Tse YT, Chan CY, MO WY, Sze ETP, Chan SMN*	*Chlorella sp.* Cultivation using Anaerobic Digestate with Chitosan Flocculation as low-cost strategies for microalgal protein and lipid production. Journal of Applied Phycology	No	Yes [Attachment 2]	Yes	No

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)*
08/2022, Hong Kong, China	Efficient biodiesel production by an integrated waste-to-resource approach., Hong Kong, China.	International Conference on Environment and Human Health: Challenges and Opportunities in the 21st Century	No	Yes [Attachment 3]	Yes	Yes
08/2023, Hong Kong, China	Exploring Low Cost Strategies for Sustainable Microalgae Cultivation and Harvesting using Wastewater and Chitosan Flocculation	International Conference on Algal Research, Application and Management	No	Yes [Attachment 4]	Yes	Yes
06/2023, Porto, Portugal	Optimizing *Scenedesmus quadricauda* Lipid Accumulation Through Different Levels of Salinity Stress	The 8th Congress of the International Society of Applied Phycology (ISAP 2024)	No	Yes [Attachment 5]	Yes	Yes

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

Research experience and new knowledge has contributed to teaching and learning.

The integrated waste-to-resource research idea in this project is an important concept in achieving sustainable development. The present study is a good demonstration of integrating different technologies to convert food waste, a type of heavy nutrient load waste, to resources that could benefit the society. Sharing of the research idea of this project to undergraduate and postgraduate students in the science field is inspiring to the students.

Through this project, the PIs gained lot of experiences in setting up bioreactor for microalgae cultivation, maintaining fish cultures, dissection of fish specimen, various kinds of chemical and biochemical analysis. These experiences and techniques furnishing the PIs' teaching, especially in laboratory classes of undergraduate science, testing and final year project courses.

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
N/A			

12. Other Impact

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

Chan SMN*, Sze ETP, Mo WY, Tam SC, Wong ESW

Patent (Hong Kong): Fish Feed Composition and Method of Production the same Application No.: 32024092362.5 [Attachment 6]

13. Statistics on Research Outputs

	Peer-reviewed Journal Publications	Conference Papers	Scholarly Books, Monographs and Chapters	Patents Awarded	Other Research Outputs (please specify)	
No. of outputs arising directly from this research project	2	3	0	1	Type	No.

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
N/A	