RGC Ref. No.:
UGC/FDS25/E16/17
(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

Synthesis of PHAs using Carbon Source from Ferrous Activated Persulfate Oxidation combined with Alkaline Fermentation of Excessive Activated Sludge

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

Research Team	Name / Post	Unit / Department / Institution
Principal Investigator	Dr. LIU Yaohui /Assistant Professor	Faculty of Science and Technology, Technological and Higher Education Institute of HK
Co-Investigator(s)	Dr. SONG Xiulan / Associate Professor	School of Environment Science and Engineering/Taiyuan University of Technology, Taiyuan, Shanxi, China
Others		

3. Project Duration

	Original	Revised	Date of RGC / Institution Approval *(must be quoted)*
Project Start Date	1 Jan 2018	N/A	N/A
Project Completion Date	31 Dec 2019	30 Jun 2020 31 Dec 2020 30 Jun 2021	24 Oct 2019 (by THEi) 26 Jun 2020 (by RGC) 14 Dec 2020 (by RGC)

Duration *(in month)*	24	30 36 42	24 Oct 2019 (by THEi) 26 Jun 2020 (by RGC) 14 Dec 2020 (by RGC)
Deadline for Submission of Completion Report	31 Dec 2020	30 Jun 2021 31 Dec 2021 31 Dec 2021	24 Oct 2019 (by THEi) 26 Jun 2020 (by RGC) 14 Dec 2020 (by RGC)

Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. To investigate the mechanisms of alkaline fermentation using persulfate salts and ferrous ion.
2. To compare the efficiency of ferrous activated persulfate oxidation combined alkaline fermentation with other common methods
3. To study the optimal dosing fraction for ferrous activated persulfate oxidation combined alkaline fermentation based on degradation of extracellular polymeric substances and dewaterability.
4. To investigate the conditions for using the digested carbon source from ferrous activated persulfate oxidation combined alkaline fermentation to produce PHAs.
5. To study the compositions and mechanical properties of PHAs produced from the digested carbon sources.

5.2 Revised objectives

Date of approval from the RGC: N/A

Reasons for the change:

1.

2.

3. ....

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)

1. To investigate the mechanisms of alkaline fermentation using persulfate salts and ferrous ion.
The objective is achieved by studying the alkaline fermentation of waste activated sludge was collected from wastewater treatment works using Peroxymonosulfate (PMS) as oxidant. The effect of PMS + pH10 on SCFAs production from WAS fermentation was investigated and compared with sole PMS and sole pH 10. It is found that PMS + pH10 enhances the SCFAs production, especially the acetic acid production with PMS dosage of 0.08 g/g TSS. PMS combined with pH10 fermentation brings positive synergy on sludge dissolution, hydrolysis and acidification, and suppresses the activity of methanogenesis.

2. To compare the efficiency of ferrous activated persulfate oxidation combined alkaline fermentation with other common methods
The objective is achieved by comparing the SCFAs production from WAS fermentation among sole PMS, sole pH 10 and PMS + pH10. It has been found that PMS + pH10 greatly enhances the SCFAs production especially under the dosage of PMS of 0.08 g/g TSS. In addition, PMS+pH10 could shorten the fermentation time to day 5 to obtain the maximal accumulation.

3. To study the optimal dosing fraction for ferrous activated persulfate oxidation combined alkaline fermentation based on degradation of extracellular polymeric substances and dewaterability.
The objective is achieved by studying the extracellular polymeric substances (EPS) degradation using Fe_3O_4 + persulfate salt to control the oxidation process. The finding showed that, with the dose of 2 g/L Fe_3O_4 and 1 mM PS at 30 °C, the EPS is greatly digested. In addition, some persistent organics, such as Triclosan, are also degraded under this condition.

4. To investigate the conditions for using the digested carbon source from ferrous activated persulfate oxidation combined alkaline fermentation to produce PHAs.
The objective is achieved by feeding fermented broth from WAS for the PHAs synthesis. The maximum PHAs content accumulated in activated sludge is 12.22wt% and 10.61wt% (proportion to VSS) when simulated pH10 fermentation broth and simulated pH10+PMS fermentation broth are substrates respectively.

5. To study the compositions and mechanical properties of PHAs produced from the digested carbon sources.

The objective is partially achieved by analyzing the portion of HB and HV fraction in the PHAs because they are two main monomers of PHAs. The PHB content of the two substrates is higher than PHV. The melting temperature of the PHAs accumulated from the study was investigated. The results showed that the more PHB contents the higher melting temperature. The mechanical analysis was not yet completed because outbreak of the pandemic affects the progress of the study significantly.

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 investigate the mechanisms of alkaline fermentation using persulfate salts and ferrous ion.	√	100%
2. To compare the efficiency of ferrous activated persulfate oxidation combined alkaline fermentation with other common methods	√	100%
3. To study the optimal dosing fraction for ferrous activated persulfate oxidation combined alkaline fermentation based on degradation of extracellular polymeric substances and dewaterability.	√	100%
4. To investigate the conditions for using the digested carbon source from ferrous activated persulfate oxidation combined alkaline fermentation to produce PHAs.	√	100%
5. To study the compositions and mechanical properties of PHAs produced from the digested carbon sources.	√	80%

6. Research Outcome

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

This project aimed to explore the feasibility of obtaining Short-Chain Fatty Acids (SCFAs) to synthesize Polyhydroxyalkanoates (PHAs) from alkaline fermentation of excess sludge combined with Peroxymonosulfate (PMS). The major findings are concluded as follows:
(1) The maximum PHA content in activated sludge is 12.22wt% and 10.61wt% (proportion to VSS) when simulated pH10 fermentation broth and simulated pH10+PMS fermentation broth are substrates respectively, and the PHB content of the two substrates is higher than PHV. The PHA production performance of the enrichment reactor when the simulated pH10+PMS fermentation broth is the substrate is better than the PHA production performance of the reactor with the simulated pH10+PMS fermentation broth as the substrate. Therefore, the simulated pH10+PMS fermentation broth was selected as the most suitable substrate.
(2) The substrate sequence of the maximum PHA content in activated sludge is: simulated pH10+PMS fermentation broth>simulated pH10 fermentation broth, 52.53wt% and 48.84wt% respectively. PHB and PHV have the same changing law as PHA, and the content of PHB under different substrates is higher than that of PHV (Figure 1).

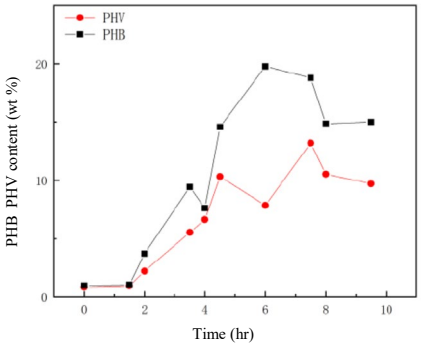


Figure 1 Typical PHB and PHV contents during PHAs synthesis process using pH10+PMS fermented broth

(3) Without adding nitrogen and phosphorus, using a simulated pH10+PMS fermentation broth as a substrate, the effects of aeration in the synthesis stage and different feeding times on the performance of PHA production were investigated. The results showed that: in the aeration rate range of 0.8-1.6L/min, the PHA content in the activated sludge increases with the increase of the aeration rate, and reaches the maximum when the aeration rate is 1.6L/min, which is 67.30wt%. The order of feeding times of the maximum PHA content is: three feedings>one feeding>five feedings, and the PHA content under the three feedings is 83.01wt%. Comprehensive analysis shows that the optimal parameter conditions for the PHA synthesis stage are: aeration rate of 1.6L/min, and three feeds for each batch.
(4) The struvite precipitation method was used to remove ammonia nitrogen and phosphate from the actual sludge fermentation broth (actual pH 10+PMS fermentation broth), and the removal effects of Mg/P and pH on ammonia nitrogen and phosphate were investigated. The best conditions obtained are: pH10, Mg/P=2.4, under these conditions, the removal rate of phosphate is 88.09%, and the removal rate of ammonia nitrogen is 71.98%.
(5) When the sludge fermentation broth from which nitrogen and phosphorus have been removed is used as the substrate, the maximum PHA content of the mixed flora in the PHA synthesis stage is only 31.98wt%, which is caused by the fact that the actual sludge fermentation broth contains a large amount of Dissolved Organic Matters (DOM).
It is recommended to control the concentration and type of DOM in the follow-up research to maintain it at a more appropriate level; improve the degradation efficiency of macromolecular organic substances and the acid production rate of sludge fermentation broth; the sludge fermentation broth

was diluted to a certain concentration and then used as the influent substrate in subsequent experiments; increasing the batch duration; increasing the load gradually; and gradually increasing the adaptability of the flora to the actual fermentation broth.

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

The production of biodegradable PHA-containing bioplastic is quite useful to both industry and the environment. However, the application of PHAs in plastic industries has been hindered due to high production costs, brittle fracture behavior due to a high level of crystallinity, poor processability, and low thermal stability in the molten state. PHAs gained particular interest since they are completely biodegradable, non-toxic and biocompatible, and are considered as green plastics for industrial application. The toughness and processability of PHB can be improved by incorporation of the hydroxyvalerate (HV) monomers in the bacterial fermentation process. While PHBV with higher HV weight ratio has higher flexibility, low crystallinity and gain improvement of the yield strength and Young's modulus of PHB with appropriate properties. With the help of the findings from this project,

Nowadays, filtration method is the most reliable technique for efficient protection from particulate matter contamination, hazardous gases and further air borne diseases in the living environment. Traditional air filtration medium (micron-scale fibers), such as melt-blown fibers, spun-bonded fibers, and glass fibers exhibit low filtration efficiency towards airborne nanoparticles with the size between 0.1 to 0.5 μm. The traditional air filtration membrane is made by non-biodegradable materials. The widely use of air filtering membrane, such as HEPA and facial masks increase the amount of plastic solid waste in the landfill sites. The PHAs were tested to be feasible to produce nano-scale air filtering membrane using the electrospinning technology. With the advantages of the facile electrospinning approach in the fabrication of PHA-based nanofiber membrane, one of the promising development of PHAs study is to investigate the effects of major electrospinning parameters in the optimization of nanofibrous membrane filter, and investigate the relationship between the fiber structure and physical properties of as-spun membrane and explore the potential application in air pollution control. Furthermore, the PHAs extracted from activated sludge could be also considered to be raw materials for producing air filtration membrane. With the optimum parameters, the pilot-scale and large-scale studies could be carried out for the production of disposable filtering materials, such as facial masks, to save the space of landfill sites and protect environments by biodegradable plastics.

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)

The Polyhydroxyalkanoates (PHAs) is one of the promising biodegradable plastics attracting lots of interests in the world. The major obstacle of large application is the high production cost, which includes mainly the carbon source cost. The findings showed that the persulfate fermentation broth could be used for the PHAs production. This significantly increases the production efficiency of PHAs from activated sludge by shortening the fermentation and hydrolyze the carbon source. The control of the dissolved organic matters in the broth is shown to be an effective way to adjust the composition of the HV and HB fraction in the PHAs. This realizes the method to produce the PHAs with desired properties for further application, such as producing air-filtering membrane. The findings from this project significantly improves the study of PHAs in saving the cost and increasing the production efficiency.

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)*						
			√	LIU, Y.H.*, SONG, X.L., CHUA, Hong, TSANG, Y.F.,	Compositions and Properties of Polyhydroxyalkanoates by Fermented Wasted Sludge as Carbon Source (Plan to submit to *Bioresource Technology*)	No	No	Yes	
			√	Xiulan Song; Yuqing Zhang; Yaohui Liu; Pei Bi; Bingqin Su	Enhanced short-chain fatty acids production from waste-activated sludge by using peroxymonosulfate combined with alkaline fermentation (Plan to submit to *Science of the Total Environment*)	2020	No	Yes	
2020				Yaohui Liu, Yanming Wang, Cheng-Hao Lee, Chi-	Synthesis of Biodegradable Plastics from Dyeing	No	Yes	Yes	Yes

				Wai Kan, Hong Chua	Wastewater and Optimization of Continuous Poly(3-hydroxybutyrate) Fibrous Membranes via Electrospinning Process (Journal of Materials Science and Chemical Engineering, 8, 71-79.)				
2021				LIU Zhan-hua, SONG Xiu-lan, LIU Yao-hui	Effect of Carbon Source Type on PHA Synthesis and Microbial Community in Activated Sludge (Accepted by China Water & Wastewater)	No	Yes	Yes	Yes

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)*
2018/ Hong Kong	Synthesis of PHAs using Carbon Source from Ferrous Activated Persulfate Oxidation combined with Alkaline Fermentation of Excessive Activated Sludge	The 3rd International Conference on Biological Waste as Resource 2018 (BWR2018)	2018	No	Yes	Yes

2019/London	Study of Synthesis of Biodegradable Plastics from Alkaline Fermented Excessive Activated Sludge	9th World Congress on Biopolymers and Bioplastics	2020	No	Yes	Yes
2019/Hong Kong	Synthesis and Adjustment of Polyhydroxyalkanoates from Wastewater Treatment Process (FYP topic supervised by the PI)	International Conference on Applied Education, Technology and Innovation (THEi AETI 2019)	2020	No	Yes	Yes
2019/Hong Kong	Study of Production of Eco- Bricks by using Ferrous Activated Persulfate Alkaline Fermented Biosolids (FYP topic supervised by the PI)	International Conference on Applied Education, Technology and Innovation (THEi AETI 2019)	2020	No	Yes	Yes
2020/Sanya	Synthesis of Biodegradable Plastics from Dyeing Wastewater and Optimization of Continuous Poly(3-Hydroxybutyrate) Fibrous Membranes via Electrospinning Process	The 10th Conference on Nanomaterials(CN 2021)	No	Yes	Yes	Yes

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

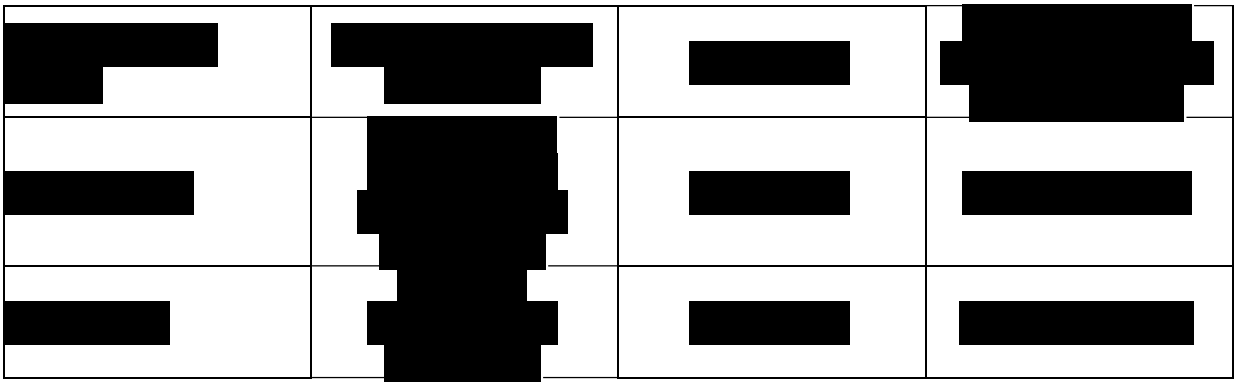
(Please elaborate)

No. The knowledge transfer will be explored for applying the PHAs to commercial products, such as air filters. This direction is still under study stage for the optimal operation parameters and feasible production process techniques. It is expected that the PHAs synthesis from activated sludge can be used to replace non-biodegradable plastics.

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]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]



12. Other Impact

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

Nil

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 (under review/preparation) 2 (published)	5	0	0	Type	No.
					M.Phil Thesis	1

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
Nil	