Ministry of Higher Education
MALAYSIA

Japan International
Cooperation Agency

The Project for Continuous Operation System for Microalgae Production Optimized for Sustainable Tropical Aquaculture (COSMOS)

Project Completion Report

March, 2022

Soka University

ED
JR
22-162

Project Completion Report

I. Basic Information of the Project

1. Country: Malaysia

2. Title of the Project: Continuous Operation System for Microalgae Production Optimized for Sustainable Tropical Aquaculture (COSMOS)

3. Duration of the Project (Planned and Actual):

Planned: 5 years from 25 March 2016 to 24 March 2021

Actual: 6 years from 25 March 2016 to 24 March 2022

4. Background (from Record of Discussions(R/D))

Japan International Cooperation Agency (hereinafter referred to as "JICA") had exchanged views and held a series of discussion through JICA Malaysia Office with the authorities concerned of Malaysia with respect to the details of "the Project for Continuous Operation System for Microalgae Production Optimized for Sustainable Tropical Aquaculture (COSMOS)" (hereinafter referred to as "the Project") and measures to be undertaken by JICA and the Ministry of Higher Education of Malaysia ("Ministry of Higher Education") for the successful implementation of the above-mentioned Project. This Record of Discussions is made pursuant to the agreement between the Government of Malaysia and the Government of Japan in the Note Verbal es of the two countries No. E.J.2015/146 and BAS0/15, and No.E.J.2015/263 and BA80/15 respectively, which amongst others, provide JICA and a competent agency of the Government of Malaysia to agree on separate arrangements which govern the details and procedures of the technical cooperation.

5. Overall Goal and Project Purpose (from Record of Discussions(R/D))

- **Overall Goal**: Mass-culture of high value microalgae is adopted by aquaculture farms towards a sustainable aquaculture industry in Malaysia and/or other aquaculture producing countries.
  1. More than two (2) types of useful compounds are produced from cultured microalgae using recycled nutrients from aquaculture pond sludge.

2. More than three (3) aquaculture farms have introduced mass culture of algae in Malaysia and/or other tropical countries.

- **Project purpose:** An energy-efficient mass-culture system of high value microalgae using recycled nutrients from aquaculture pond sludge is established.

  1. Annual algal biomass production of one (1) tonne or more per hectare is achieved.

  2. Biomass yield per giga joule of two (2) times or more than the conventional raceway system is achieved for two (2) indigenous microalgae species.

  3. More than one (1) tonne of aquaculture pond sludge is reduced for every ten (10) kilograms algal biomass production (dry weight basis).

6. Implementing Agency

- Malaysian side: Universiti Putra Malaysia (UPM), Universiti Malaysia Terengganu (UMT), Universiti Selangor (UNISEL)
- Japanese side: Soka University (SU), The University of Tokyo (UT), Tokyo Institute of Technology (TITech), National Institute for Environmental Studies (NIES)

II. Results of the Project

1. Results of the Project

1-1Input by the Japanese side (Planned and Actual)

Dispatch of Experts (Planned)

- Long-term Expert: Project Coordinator
- Short-term Expert:
  - Planktonologist/ Project Leader
  - Phycologist
  - Natural organic chemist/Soil chemist, GC-MAS
  - Analytical chemist, LC-MAC
  - Waste treatment scientist
  - Chemical engineer/ Bioreactor engineer
  - Water chemist
  - Microbiologist
  - Photobiologist
  - Natural product chemist

Dispatch of Experts (Actual)

Details are described in “Dispatched Experts” of the ANNEX 1.

- Long-term Expert:
  - Project Coordinator (Ms. Mari Miller)
  - Project Coordinator (Mr. Masaru Yamada)

- Short-term Expert:
  - Planktonologist 1/ Project Leader (T. Toda)
  - Planktonologist 2 (M. Hirahara)
  - Planktonologist 3 (Y. Takayama)
  - Phycologist 1 (K. Takahashi)
  - Phycologist 2 (M. Sato)
  - Phycologist 3 (T. Katayama)
  - Phycologist 4 (M. Ohtake)
  - Advisor/ Phycologist 4 (K. Furuya)
  - Microbiologist (N. Kurosawa)

- Photobiologist (V. Kuwahara)
- Natural product chemist (M. Watanabe or Tominaga), Watanabe Oyster Laboratory
- Natural organic chemist/Soil chemist, GC-MAS
- Analytical chemist 2 LC-MAS
- Water chemist 1 (A. Imai)
- Water chemist 2 (K. Komatsu)
- Chemical engineer/Bioreactor engineer 3 (T. Matsuyama)
- Chemical engineer/Bioreactor engineer 4 (J. Ida)
- Waste treatment scientist 1 (K. Nakasaki)
- Waste treatment scientist 2 (M. Koyama)
- Waste Treatment Scientist 3 (T.N.M. Quyen)
- Electron Microscopist (M. Kuwata)

Machinery and equipment (Planned)

- Liquid chromatography-mass spectrometry
- Liquid fractionation system
- Auto analyzer for inorganic macro nutrients
- High performance liquid chromatography
- TOC Analyzer
- Continuous flow centrifuge
- Portable gas analyzer (CH_4, CO, CO_2, O_2)
- Fluorescence Multi Plate reader
- Inverted fluorescence microscope imaging system
- Gas chromatography FID
- Fluorescence microscope imaging system
- Ultrapure water equipment
- Freeze drier
- Environmental water quality sensor
- Stereo microscope
- Elemental analyzer
- Indoor experimental small- scale reactors
- Outdoor scaled-up bag reactor unit system

Machinery and equipment (Actual)

Details are described in “Handover Equipment, Procured Equipment, Certificate” of the ANNEX 1.

Training in Japan (Actual)

Details are described in “Training in Japan and Abroad” of the ANNEX 1.

Workshop

Details are described in “Seminar, Meeting and Workshop” of the ANNEX 1.

Necessary expenses

Details are described in “JICA's Expenditure” of the ANNEX 1.

1-2 Input by the Malaysian side

Details are described in “Land, Building, Office, and Facilities provided by the Malaysian side” of the ANNEX 1.

- Services of the Malaysian Counterpart personnel and administrative personnel
- Suitable office space with necessary equipment
- Supply or replacement of machinery, equipment, instruments, vehicles, tools, spare parts and any other materials necessary for the implementation of the Project other than the Equipment provided by JICA
- Running expenses necessary for the implementation of the Project (in the form of matching fund)

1-3 Activities

- **Activity PO. 1-1: Collect microalgae from various inland and coastal water bodies in Peninsular Malaysia.** ***This activity has been completed*** and the cultures are maintained in the laboratories with routine check-up.

- <u>Activity PO. 1-2:</u> **Screen, isolate and purify the collected microalgae. *This activity has been completed*.** A total of 212 isolates were purified and screened for high lipids/carotenoids using Nile red fluorescence dye (Katayama *et al.*, 2020, 2019, 2018; Kasan *et al.*, 2020; Zakaria *et al.*, 2020). Ten (10) isolates were selected as high-value microalgae candidates.

- <u>Activity PO. 1-3:</u> **Characterize microalgae for high contents of valuable compounds. *This activity has been completed*.** Second screening of seven (7) isolates was conducted for accurate quantification of useful compounds using GC and HPLC. In addition, six (6) isolates from UMT and UPM identified earlier were also used in the second screening (Khatoon *et al.*, 2018a, 2018b, 2017; Rahman *et al.*, 2020, 2017, *under review*; Tiong *et al.*, 2020a, 2020b). As a result of the second screening, two indigenous microalgae species, *Thalassiosira weissflogii* and *Isochrysis galbana*, have been chosen as the high value microalgae with high contents of lipid and carotenoid. They are now used by Themes 2, 3 and 4.

- <u>Activity PO. 1-4:</u> **Conduct incubation experiments using variable growth conditions. *This activity has been completed.*** Experiments with selected isolates, *T. weissflogii* and *I. galbana*, have proceeded for determination of the optimal growth in culture conditions. Experiments by manipulating starvation of nitrate and phosphate, and different salinity levels have been completed for the two selected isolates. For *I. galbana*, nitrate starvation (¼ from the Control) has been shown to reduce the growth, but phosphate starvation (¼ from the Control) increases growth. For salinity, 20 and 25 ppt have been shown to have the most profound effect on the growth of *I. galbana*. Biomass at 25 ppt increased more than 20% as compared to the Control (30 ppt). For *T. weissflogii*, reduction of nitrate significantly reduced the growth. However, reduction of phosphate does not affect the growth *of T. weissflogii*. Low salinity enhances the growth and the biomass at 20 ppt is increased more than 40% as compared to the Control (30 ppt).

- <u>Activity PO. 2-1:</u> **Conduct incubation experiments under controlled culture conditions to enhance productivity of useful compounds. *This activity has been completed.*** Establishment of high-lipid/carotenoid production culture conditions (Afifudeen *et al.*, 2021a, 2021b; Loh *et al.*, 2021; Tachihana *et al.*, 2020; Tega *et al.*, 2021; Teh *et al.*, 2021, 2019; Yusof *et al.*, 2021) is now currently proceeding for the selected isolates under variable conditions. Starvation of nitrate and phosphate and different salinity had been tested for the two selected isolates. For *I. galbana*, nitrate reduction does not affect the fatty acid compositions, but the reduction of phosphate (¼ from the Control) significantly increases DHA production For *T. weissflogii*, both nitrate and phosphate starvation significantly reduced the fatty acid compositions, especially for EPA. Meanwhile, for salinity treatments, 20 ppt has been shown to increase the production of EPA as compared to the Control.

- **<u>Activity PO. 2-2:</u> Characterize and quantify the useful compounds in cultured microalgae as in 2-1. *This activity has been completed.*** For *I. galbana*, DHA production has been increased more than 20% by reducing phosphate (¼ from the Control). Productivity of carotenoids is currently determined for the cultured microalgae as in 2-1.

- **<u>Activity PO. 3-1:</u> Collect soil and sludge from various locations in Malaysia**. ***This activity has been completed*** for all 9 sites in Peninsular Malaysia. See the report in Term 7 for details. For the application study at the demonstration site, additional soil samples from Singa Island, Timun Island in Pulau Langkawi Forest Reserve and Raja Musa Peat Swamp soil is postponed within Term 11.

- **<u>Activity PO. 3-2:</u> Study soil and sludge extraction methods.**

This activity has been completed where seven (7) extraction methods were tested (Yaacob *et al.*, 2021; Zamri *et al.*, 2021).

- **<u>Activity PO. 3-3:</u> Examine and optimize fractionation method of soil and sludge extracts. *This activity has been completed***. In addition to the resin fractionation technique, the molecular size fractionation method by using ultrafiltration membranes has been established. See the report in Term 8 for details (Kawasaki *et al.*, 2016a, 2016b).

- **<u>Activity PO. 3-4:</u> Conduct plate culture technique and examine the growth-promoting fractions from soil and sludge extraction. *This activity has been completed.*** Plate incubation experiments using microalgal strains selected by Theme 1 have been completed in Term 9. The growth promoting effects of the UNISEL-made 14 kinds of soil extracts (S.E.) obtained under a condition of 121°C for 1hour (2 times) on those microalgae were examined (Arumugam *et al.*, *in press*, 2021, 2020). Some soil extracts could enhance the growth of microalgae by the plate incubation experiments so far. Based on these results, samples of L-a and L-b soil extracts were prepared and sent to SU for scaled-up experiments using bubble-column reactors (10L) after obtaining the permission of The Plant Quarantine in Japan. Sample from Raja Musa Peat Swam (R-1) which enhanced the algae growth was extracted and ready to send for outdoor photo bioreactor test at UPM demonstration site.

- **<u>Activity PO. 3-5:</u> Examine the chemical characteristics of the growth-promoting fractions and their effects on the physiological responses of microalgae. *This activity has been completed***. The growth-promoting effects of the different dissolved organic matter (DOM) fractions were examined by the advanced plate incubation experiments with adding each fractionated fractions of the soil extracts to the growth media (Conway). The soil extract solutions were fractionated into humic, non-humic, low-molecular-weight, and high-molecular-weight fractions and the efficacy tests of *Chlorella sorokiniana*, *Oocystis heteromucosa*, and *Thalassiosira weissflogii* were conducted. The additions of the humic and low-molecular-weight fractions were consistently associated with the greater growth-enhancing effects. Characterization of DOM (UV absorbance, molecular size distribution, and fluorescence analysis) in all the S.E. imported from UNISEL has been completed (Komatsu *et al.*, 2020, 2019a, 2019b; Shimotori *et al.*, 2016). In addition, since the highest iron concentration in L-b which was one of the most effective soil extracts was observed, it may suggest that iron supply is also one of the most conceivable factors for the algal growth enhancement. The chemical composition of soil organic matter (SOM) can be an important determinant of the nutrient supply capacity of soils.

- **<u>Activity PO. 4-1:</u> Develop culture methods of selected microalgae in a small scale photobioreactor. *This activity has been completed***. Culture experiments on *Spirulina* and *Chlorella* were carried out by using the laboratory-scale photobioreactor.

- **Activity PO. 4-2: Develop scaled-up novel photobioreactor for outdoor mass culture. *This activity has been completed***. To evaluate how agitation frequency affects the biomass production and pigment contents of diatom on high-density culture using an outdoor scaled-up bag reactor, semi-continuous incubation of *C. gracilis* was conducted under different agitation conditions. High agitation frequency was more effective than the low agitation frequency in terms of the estimated biomass production per power input.

- **<u>Activity PO. 4-3:</u> Develop a multi-sequence system of operational photobioreactors. *This activity has been completed*.** Due to flood damage and some complications with the demonstration site photobioreactor system, we reconstructed the concrete ground of the site and made modifications to the multi-sequence photobioreactor system and seawater storage tank, respectively. The outdoor-scaled-up multiple bag reactor system (a multi-sequence system of operational photobioreactors) has been developed in consultation with Japanese chemical manufacturers. From Term 9, we organized a cross-functional team to develop the novel photo-bag reactor for implementation. The revised designs were developed, and the prototype was installed at SU to monitor the operational performance in March 2021.

  The laboratory-scaled cultivation using selected microalgae such as *Chaetoceros gracilis*, *Thalassiosira weissflogii, Isochrysis galbana* and *Tetraselmis tetrathele* has been continuously carried out. For *I. galbana* two-phase culture was conducted with different supply rates of total ammonium nitrogen to achieve a high-density culture under continuous low-supply of ammonium as a nitrogen source (Goto, 2018, 2020; Yago, 2020). *Tetraselmis tetrathele* was cultured in different ammonium nitrogen concentrations to investigate the effect on growth rate, photosynthetic efficiency, and pigment contents to high ammonium nitrogen concentrations (Farahin, 2021; Farahin et al., 2021). These studies clarified that these selected microalgae are highly tolerant to high ammonium nitrogen. The suitable cultivation method for diatoms was established by controlling the agitation frequency and the supply rate of carbon source. Growth promoting soil extract from UNISEL was also being used to grow *T. weissflogii* and *I. galbana* using bubble column reactor.

  The outdoor-scaled-up multiple bag reactor system (a multi-sequence system of operational photobioreactors) was installed in SU to do the trial run in July 2021 and was introduced at the demonstration site in October 2021. The integration of system parts at the demonstration site and continued experiments were completed in March 2022.

- **<u>Activity PO. 5-1:</u> Establish a technology for recovery of nutrients from aquaculture pond sludge. *This activity has been completed*.** TiTech is investigating the NH_3 recovery potential of shrimp aquaculture sludge using laboratory and bench-scale aerobic fermentation systems (Kakiuchi, 2021; Koyama *et al.*, 2022, 2020, 2019, 2018; Nakagawa, 2021, Syazni *et al.*, *under review*). UPM conducted a test-run by outdoor large-scale nutrient recycle system with the mixture of shrimp sludge and cow dung and confirmed that thermophilic composting progresses. Test-run with 100% sludge was conducted using a new sludge. Also, TiTech is investigating phosphate recovery and salinity removal from the fermentation solid residue (*i.e.* compost) of shrimp pond sludge for culturing microalgal biomass and desalinating the compost for agricultural application. UPM started to examine plant growth effect of the shrimp sludge compost, investigating the growth of some halo-tolerant and high-value crops (mango and fig).

- **<u>Activity PO. 5-2:</u> Establish a technology to utilize ammonia and phosphorus as a low-cost alternative nutrient source. *This activity has been completed***. TiTech, SU and UPM started to optimize a NH_3 trapping/storage/supply method towards the efficient utilization of nutrients emitted from sludge composting, for culturing high-value microalgal biomass. Feasibility of this system has been investigated. TiTech and UPM completed the modification and the operation test of the Outdoor large-scale nutrient recycle system (4 m^3) at the demonstration site (pilot-scale sludge composting system with NH_3 recovery unit, connected with a bag-reactor microalgae cultivation system) for improving the system safety and to install the abovementioned gas absorption system. Test-run by empty Outdoor large-scale nutrient recycle system was conducted and confirmed that it functions correctly. Test-run with sludge was conducted using a new sludge. The system operation needs to be optimized for the safe and sustainable operation over a long time period. TiTech and UPM completed designing and constructing the sludge de-watering facility to pretreat large amounts of sludge, in order to operate the pilot-scale composting system.

  After improving the system, the plan was to conduct the main operation using sludge from aquaculture ponds. However, due to a combination of regional flooding, delays in shrimp farming, and pandemic related events, sludge collection was not conducted. Therefore, the main operation was conducted using cattle manure, which has similar properties to sludge, and the results showed that 66.3 mol of ammonia gas could be volatilized and recovered per ton of dry weight of the raw material. The nitrogen content of the pond sludge was 3.4 times higher than that of the cattle manure used. Therefore, it is estimated that up to 222.6 mol/ton of ammonia (equivalent to a theoretical algae yield of 29.7 kg-algae/ton if converted to algae biomass, exceeding the project's target of 10 kg-algae/ton) can be recovered using the sludge.

- <u>**Activity PO. 6-1:**</u> **Analyze environmental impact and economic value of the production system by LCA and LCC. *This activity has been completed*.** The system scale was determined as "40 shrimp ponds (common scale for small-scale shrimp farm)" to economically treat the sludge. We estimated that 10 tons (dry weight) of sludge are generated every three days at this scale. Therefore, we designed an aerobic fermentation plant to recover ammonia from the sludge and a floating photobioreactor to cultivate a large amount of *Isochrysis galbana*, to evaluate the life cycle cost of this integrated system.
  The environmental impact of the integrated system was estimated based on the carbon and nitrogen concentrations in the sludge and the amount of CO_2 generated during the fermentation process and degradation of the compost in the environment.

- <u>**Activity PO. 6-2:**</u> **Monitor biological production and environmental properties in aquaculture pond installed with algae production system. *This activity has been completed.*** Proof of concept was achieved by constructing the demonstration site.

2. Achievements of the Project

2-1 Outputs and indicators

1. High value microalgae are isolated and suitable growth conditions in their life cycle and physiological attributes are determined.

Output: 1-1. More than two (2) indigenous microalgae species with high contents of fatty-acid, antioxidant and other useful compounds are identified.

Achievement: 1-1: Two indigenous microalgae species, *Thalassiosira weissflogii* and *Isochrysis galbana*, have been chosen as high value microalgae with high content of lipid and carotenoid.

Output: 1-2. Optimum growth conditions are determined for selected microalgae species.

Achievement: 1-2: This incubation under various environmental conditions such as light, temperature, nutrients, salinity **has been completed**.
Optimum growth conditions are currently determined for the two selected microalgae species, *Thalassiosira weissflogii* and *Isochrysis galbana*. For both microalgae species, 20 ppt has been shown to have the most profound effect on their growth.

2. Production of useful compounds of selected microalgae is enhanced (by controlling culturing conditions and life-cycle).

Output: 2-1. Suitable culture conditions for increasing productivity of useful compounds are determined for selected microalgae species.

Achievement: 2-1. This incubation under various environmental conditions such as light, temperature, nutrients, salinity **has been completed**.
High oil production culture conditions were established for *Chlorella vulgaris* and *Messastrum gracile* microalgae model. Based on these findings, suitable culture conditions for inducing production of useful compounds are currently determined for the two selected microalgae species, *Thalassiosira weissflogii* and *Isochrysis galbana*. For *T. weissflogii*, the best culture

conditions are full strength phosphate and nitrate in 20 ppt salinity to induce EPA content.

Output: 2-2. Productivity of useful compounds is increased by twenty (20) percent as compared with the standard growth condition.

Achievement: 2-2. ***This activity has been completed.***
Productivity of carotenoids was currently determined for the cultured microalgae as in 2-1. For *I. galbana*, DHA production has been increased more than 20% by reducing phosphate (1/4 from the Control).

3. Efficient microalgae culturing technique utilizing growth-enhancing substances extracted from soil and sludge is proposed.

Output: 3-1. Optimal methodologies for extraction and fractionation of growth enhancing substances are identified.

Achievement: 3-1: By the development of a fully automatic fractionation system, the optimal methodologies for fractionation have been established. Additionally, the methodology of the molecular size fractionation by ultrafiltration with centrifugation has been established. Those two established fractionation techniques were applied to OVI. 3.2.

Output: 3-2. Using plate culture technique, relationships between enhancing effect on algal growth and chemical characteristics of soil extraction fraction are clarified.

Achievement: 3-2: Using plate culture techniques, the growth effects on some algal species by the soil extracts have been clarified. Growth promoting effects of soil extracts have been determined on selected microalgae provided from the NIES culture collection. In cooperation with Theme 1, the growth promoting effects of soil extracts on high-valued microalgae have been investigated and then observed. Furthermore, the effective soil extract fractions were found by the combined experiment of plate incubation and fractionation. The role of each fraction (hydrophobic/hydrophilic) of soil extract was examined and the synergetic/offset effects of the fractions were determined by the advanced plate incubation experiments.

Through the EEM-PARAFAC (Excitation Emission Matrix combined with Parallel Factor Analysis), it was suggested that either humic-acid like or marine-humic like fluorophores had the significantly correlated with the specific growth rate. Furthermore, it was suggested that iron is one of the most conceivable factors for the algal growth enhancement.

Out of the 11 sites, 6 sites were completely examined for the 11 species of microalgae. The undiluted fraction of M-a samples was provided to UPM for further testing in small- and large-scale bioreactors. Characterization of the soil extracts with growth promoting effects was completed. Pristine soils dissolved organic matters and bacterial community profiling via shotgun metagenomics approach was successfully carried out on Royal Belum samples.

4. Closed photobioreactor is scaled up for outdoor culture.

Output: 4-1. An experimental small-scale CRADLE reactor whose biomass yield is two (2) ton per giga Joule, which is twenty (20) times higher than conventional reactors, is developed.

Achievement: 4-1: ***This activity has been completed*** in 2018

Culture experiments on Spirulina and Chlorella were carried out by using the laboratory-scale photobioreactor and achieved the yield of more than 2 Ton-Dry biomass per GJ (Term 3), which is around 20 times high energy efficiency compared to the value in a current raceway pond. The laboratory-scale photobioreactor has been installed since 2016 (Term 1). Twelve (12) column reactors which control temperature and aeration rate were designed and installed in the laboratory (Term 2).

Output: 4-2. Scaled-up bag CRADLE reactor of more than one (1) square meter with an automated driving system, which can support a culture period of more than two (2) months, is developed.

Achievement: 4-2: ***This activity has been completed*** in 2019 (Term 7)

The scale-up novel bag photobioreactor system was installed in a UPM

(Term 4) laboratory, and the culture experiment on indigenous Chaetoceros strain, which produces high value compounds, was operated continuously for more than 2 months (Term 7).

Output: 4-3. More than two (2) species of microalgae are cultured in sequenced bag reactors.

Achievement: 4-3: ***This activity has been completed*** in March 2022. The multi-sequence system of operational photobioreactors was installed in October 2021. The two species identified by Theme 1, *Isochrysis galbana* and *Thalassiosira weissflogii*, were experimentally tested.

5. Technologies for recovery of nutrients from aquaculture pond sludge and utilization of the nutrients for microalgae production are developed.

Output: 5-1. Technology for ammonia and phosphorus recovery from aquaculture pond sludge is established.

Achievement: 5-1: Achieved in lab-scale experiments (Term 5-6). Bacillus sp. strain R2 was successfully isolated from sludge compost sample. We have found the optimum fermentation temperature, prospective sludge-degrading microorganism, and liming effect for enhancing NH_3 recovery (Term5).
The ammonia production potential of shrimp sludge was 5.4 kg-N per one ton of sludge. This is sufficient for the project goal "production of 10 kg dry weight of algal biomass (=0.9 kg-N) from one ton of sludge".
$Ca(OH)_2$ dosing operation to enhance ammonia volatilization was established without inhibiting microbial activity, both in lab-scale and bench-scale.
Sludge sampling in Malaysia (Term 2 and 3)
Designed a system for recovery of nutrients from aquaculture pond sludge (Term 3).
Set up the outdoor medium-scale nutrient recycling system (Bench-scale nutrient recycling system) (Term 4).
Delivered the outdoor large-scale nutrient recycling system (Pilot-scale nutrient recycling system) to the demonstration site (Term 8).

The modification of the Outdoor large-scale nutrient recycle system was completed (Term 9-10).

Output: 5-2. Technology to utilize ammonia as a low-cost alternative nitrogen source is established.

Achievement: 5-2: Trial run completed; full operation planned at the demonstration site.
Proposed a two-stage gas absorption system to optimize a NH_3 trapping/storage/supply method.
Installed the sludge de-watering facility to pretreat large amounts of sludge at the demonstration site.
The trial run of the two-stage gas absorption system and sludge de-watering facility were conducted and progress of thermophilic composting was confirmed (Term 11).

6. Economic profitability and environmental compatibility of the system are verified.

Output: 6-1. Calculated life cycle revenue generated from a production unit exceeds life cycle cost.

Achievement: 6-1: The calculation of life cycle revenue generated from a production unit exceeds life cycle cost was achieved (Term 11).
A detailed Life Cycle Cost (LCC) assessment was completed in March 2022 (Term 12). This analysis suggests that application of the integrated project technologies (utilized at the demonstration site) for 40 ha scale shrimp farm (1 ha plastic-lined shrimp pond x 40, which is a common scale in Malaysia) with plastic lining in Malaysia will recover the initial cost within 1-year of operation dependent on commercial cost of different microalgae species. The LCC analysis revealed that the developed system is energy-saving. Also this system is environmental-friendly calculated by LCA analysis.

Output: 6-2. The system is proven not to negatively affect the current aquaculture production.

Achievement: 6-2: The evaluation of negative effects of no leakage of culture and toxic chemicals of the integrated system to current aquaculture production was completed in March 2022 (Term 12).

Output: 6-3. Total CO_2 emission by Life cycle assessment is reduced as compared to conventional sludge treatment methods.

Achievement: 6-3: The calculation of total CO_2 emission was achieved (Term 11). Scenario simulations and evaluation system boundaries were conducted in March 2022 (Term 12). The amount of carbon and nitrogen fixed in microalgae biomass and compost in the integrated system was estimated to be 55% and 44%, respectively, of the carbon and nitrogen in sludge that was previously discharged directly into the aquatic environment.

2-2 Project Purpose and indicators

Purpose 1: Annual algal biomass production of one (1) tonne or more per hectare is achieved.

Achievement 1: Production of one tonne per ha (1t/ha) has been achieved based on the scale-up novel photobioreactor (in-door photobioreactor) (Term 4).

Purpose 2: Biomass yield per giga joule of two (2) times or more than the conventional raceway system is achieved for two (2) indigenous microalgae species.

Achievement 2: The biomass yield per giga joule of two (2) times the conventional raceway system by the outdoor photobioreactors was achieved at 2.60 tons/GJ (Term 12).

Purpose 3: More than one (1) tonne of aquaculture pond sludge is reduced for every ten (10) kilograms algal biomass production (dry weight basis).

Achievement 3: One (1) tonne of aquaculture pond sludge is reduced for 11.8 kilograms algal biomass production (dry weight basis) based on completed analysis (Term 12). The integration of system parts at the demonstration site and proof-of-concept were completed in March 2022 (Term 12).

3. History of PDM Modification

- During the six years of the project, some members were replaced, moved, or retired. For more information on these, please refer to “Monitoring Sheet”.
- Due to the extreme erosion damage at the demonstration site, extension of project period is currently under consideration by JICA.
- In the 5th JCC meeting held on 28th August 2020, JICA and Malaysian authorities agreed that the Project’s duration is to be extended for one (1) year due to the landslide (erosion) problem at the Project’s Demonstration Site and the current Covid-19 pandemic as follows:
  - The duration of the project on the Project Design Matrix (PDM)
    Before amendment: 5 years (April 2016 – March 2021)
    After amendment: 6 years (April 2016 – March 2022)
  - Project Design Matrix (PDM) and Plan of Operation (PO)
    The revised PDM and PO herewith for the extended period is stipulated

4. Others

4-1 Results of Environmental and Social Considerations (if applicable)

(1) Examples of Deployment

(i) A facility to store useful algae strains and other materials collected in this project was established in the “SATREPS – COSMOS Research laboratory” at UMT in July 2017. It is expected that this facility will continue to serve as a seed bank (library) for microalgae in Malaysia after the completion of this project.

(ii) In order to disseminate the knowledge and experience of this project to researchers in Africa, the project applied for and was accepted by the Private University Branding Project of the Ministry of Education, Culture, Sports, Science and Technology (PLANE3T project, task name: PLANkton Eco-engineering for Environmental and Economic Transformation, 2017 - 2022).

(iii) Following the PLANE3T project, application for the JST-JICA SATREPS project was selected (SATREPS-EARTH; The project for Eco-engineering for Agricultural Revitalization Towards Improvement of Human Nutrition (2021 - 2026).

(iv) Several additional research projects based on COSMOS research and development were selected for funding, including the competitively funded Grant-in-Aid for Scientific Research, JSPS (KAKENHI), the Sasakawa Science Research Grant and other research funds.

(2) Implementation of social initiatives

(i) The UPM has already budgeted for a demonstration site that will serve as a showcase (proof-of-concept) for companies, enabling smooth operation. It is expected to play an integral role in social implementation phase of the project.

(ii) The "BLUE-SEED" program (Business Logistics, Universal research, development and Enterprise, Social Engagement, Education and Demonstration) aims to maintain and utilize the provided equipment and demonstration site after the completion of the COSMOS project, conduct demonstration experiments and product development in aquaculture ponds for social implementation, build a business model, and establish a venture company originating from this project. The BLUE-SEED Program is led by Dr. Muhammad Fadhil Syukri Ismail of UPM and has already received a modest operating budget for FY2022.

(iii) The project leader and the counterpart principal investigator, Prof. Fatimah Md. Yusoff, are negotiating with three Malaysian aquaculture companies (Blue Archipelago Sdn Bhd, Hannan Sdn Bhd, and RE Millenium Sdn Bhd) to introduce this production system. The company has concluded an MOU with Blue Archipelago, the largest of the three, and the feasibility of introducing this system is extremely high.

(iv) The local government in Nagasaki Prefecture (Shin-Kamigoto Town) approached and requested cooperation for this project, and the local government applied for the regional revitalization promotion grants from the Cabinet Office in FY 2018 for the SATREPS-COSMOS project using mass cultivation of microalgae (project name: "6th industrialization project using large-scale cultivation of high-value microalgae"). As a result, the project was evaluated as a distinctive example of efforts and was officially adopted in March 2019. In March 2020, the venture company supported by the project decided to use the patent for the microalgae reactor, and the technology transfer was completed.

(v) In order to advertise the international collaboration, an international conference (COSMOS International Conference: CIC) will be held in Kuala Lumpur, Malaysia, jointly organized by Soka University, UPM, JICA, and JST to highlight the research results obtained through the project and to discuss the future prospects of the project. The objectives of this international conference are as follows: (1) to provide a forum for the exchange of information on advanced findings and methodologies obtained through the project activities to enable knowledge sharing and technology transfer, (2) to promote joint research and development by industry, government, and academia, and (3) to apply for international large-scale joint research funds.

4-2 Results of Considerations on Gender/Peace Building/Poverty Reduction (if applicable)

The environmental-friendly process with a novel microalgae mass culture system using recycled nutrients from aquaculture waste will be a useful model for adaptation in many developing countries for the sustainable development of the aquaculture industry. The aquaculture with microalgae production will lead to greater incomes for local people, which in time will help to reduce poverty and build peaceful societies.

III. Results of Joint Review

1. Results of Review based on DAC Evaluation Criteria

Relevance: evaluated as “extremely high”

(1) Malaysia, as one of the 12 mega-diversity countries in the world, has a plan in its National Biodiversity Strategy (1998) to develop "new industries based on biodiversity functions" into a global industry. The COSMOS project, which contributes to the search for useful indigenous species and their commercialization, meets the needs of the counterpart country.

(2) The National Biodiversity Strategy and Action Plan considers the wetlands surrounding mangrove forests as an important habitat for the nation's major protein sources (fish and crustaceans), and protects mangrove forests and surrounding wetlands from the perspective of food security. The COSMOS project helps prevent the destruction of mangrove forests due to organic pollution and enables a safe and stable food supply that meets HACCP and other standards.

(3) Malaysia's national policy, "Vision 2020," recognizes the socioeconomic gap in Malaysia as a challenge and important national issue. The sustainable system in this project, which can be used in coastal areas where many of the economically challenged population live, will contribute to reducing regional socioeconomic discrepancies.

(4) The development of young researchers (capacity development) through this project will contribute to the need to improve the level of higher education, one of the six national priority achievement sectors in the national policy "Vision 2020".

Coherence: evaluated as “high”

(1) This project provides leading technology for environmental restoration and biological production of high value-biomass resources, and will make a significant contribution to the promotion of (1) "Reform of Green Innovation" and (2) "Reform of Life Innovation" in the "Realization of Sustainable Growth and Social Development for the Future" of the 4th Science and Technology Basic Plan.

(2) The establishment of mass production technology for algae biomass will provide a significant link to (3) "Strengthening Japan's industrial competitiveness (development of world-leading green technology)", (4) "Contributing to solving global issues" and (5) "Promoting research and development to solve common environmental problems in the Asia Pacific region" in the "Responding Japan's Key Challenges" section.

(3) This project will promote the development of human resources and education through joint research with foreign countries conducted by a wide range of professionals from industry, government, and academia, including (6) "development of human resources who can be active in diverse fields," and (7) "development of creative and productive researchers”.

(4) In the "Comprehensive Strategy for the Rebirth of Japan," the project is deeply related to the keywords for Japan's rebirth: (8) "Green", (9) "Life", and (10) "Sixth Industrialization of Agriculture, Forestry, and Fisheries”.

<u>Effectiveness</u>: evaluated as “extremely high”
The project was effective to produce core results in each of the following research themes: 1) search for useful microalgae, 2) search for natural growth-promoting substances, 3) development of a novel photobioreactor, and 4) establishment of nutrient recovery technology and recycling processes, contributing to solving the problems originally targeted. One-hundred percent (100%) of the original project plan was implemented in March 2022.

<u>Efficiency</u>: evaluated as “extremely high”
Due to the COVID-19 pandemic, the project term was extended for one year upon approval at the JCC meeting in 2021. Although travel restrictions were imposed by the Japanese and Malaysian governments and thus research activities were restricted, as shown in the "Lessons Learnt" section below, four Japanese researchers were able to travel to Malaysia in latter half of FY2021. With the cooperation of Malaysian researchers and students, the research activities of the project were completed within the planned term (***Ratio to plan: 100%***). Project expenses were almost executed as planned (***Ratio to plan: 100.4%; as of 24 March 2022***). As shown in the section on Effectiveness, ***100% of the project***

outputs were achieved as planned. The inputs (costs and terms) of this project could be evaluated as highly efficient with regard to the outputs.

Impact: evaluated as "extremely high"

(1) Overall Project Output: In terms of capacity development and research outputs, the COSMOS project has produced approximately 15 Master's and 8 Doctoral graduates, 14 award recognitions, 10 research (SOP) manuals, conducted 16 research workshops and seminars, >17 media and press releases, 8 patents, and >100 peer-reviewed publications accepted and published.

(2) Research theme 1: More than 100 strains were Isolated by the research team and more than 20 useful microalgae were identified in terms of growth and high valued substances, allowing for discovery of new bio-resources of high economic value indigenous to Malaysia. In view of the findings, the series of studies conducted by this research group has a high impact as it has provided important findings that serve as a basis for industrial utilization of microalgae indigenous to Malaysia.

(3) Research theme 2: The research group demonstrated the availability of soil extract as a nutrient booster source, which is a high international benchmark in this research field. The research results obtained are the first findings in the region to reveal that the humic and low molecular weight fractions of organic matter with growth-promoting effects are useful for marine microalgae production. In the future, the results will lead to the creation of new artificial culture media for many difficult-to-culture microalgae.

(4) Research theme 3: The culture system developed by this research group consists of low-cost and durable plastic bags installed in the water, preventing excessive temperature increases in culture medium under tropical conditions, and allows energy-saving operation by intermittent agitation using a motor instead of energy-intensive aeration. The culture system developed are easy to transport and install because they are lightweight and compact, and are expected to be horizontally deployed in various countries and regions with vast surface-water area, thus contributing to the spread of mass cultivation of microalgae worldwide.

(5) Research theme 4: This research group has succeeded for the first time in the world an efficient recovery of nitrogen source as ammonia gas from organic sludge discharged from shrimp farms through aerobic digestion using a simple process. The nitrogen source, which is a clean gas, can be used for biomass production, opening the way for the production of supplements and medical materials that can be used for human consumption. The mass cultivation technology of microalgae is expected to play an important role in the construction of a recycling-based society as an environmental restoration technology coupled with CO_2 fixation by combining it with new technologies and other elemental technologies such as composting, aerobic treatment, and anaerobic treatment (methane fermentation) technologies.

(6) This resource recycling system can promote carbon neutrality by utilizing the excellent blue-carbon function (carbon fixing capacity) of microalgae as well as ecosystem conservation, and will contribute to the carbon neutrality goals set by the Japanese and Malaysian governments by 2050.

2. Key Factors Affecting Implementation and Outcomes

(1) Cost-reduction of the total process and its components is key to effective implementation and outcomes as follows:
- The current bag reactor system was researched and developed in Japan. The microalgae culture system developed in this project consists of several components, and almost all of the component parts and materials are obtainable locally. If these parts and materials are purchased locally, the cost can be reduced by a quarter for pumps and motors and by a tenth for floats and rafts, compared to the current system at market prices. The price of the soft plastic film that composes the bag reactor is expected to be about a quarter of the price, assuming that the materials are purchased and manufactured locally.

(2) Educating, training and establishing protocols for the usage of the system by local aquaculture farmers are key to implementing this novel system to the local community.

(3) Commercialization of algae biomass is a key issue in "Business &

Commercialization," one of the four pillars of our exit strategy (BLUE-SEED Program). In order to promote this enterprise, the creation of a business model and supply chain with high profitability based on LCA and LCC analysis, and the participation of private companies with the patented innovative technologies are the keys to implementation.
- UPM has a program to provide grants for the establishment of venture companies, which are expected to be established by the end of FY2022.

(4) Continued exchange with Malaysian universities and researchers and acceptance of international students

3. Evaluation on the results of the Project Risk Management

(1) Erosion damage due to heavy flooding
The concrete base of the UPM demonstration site was damaged by erosion in FY2019 caused by the heavy flooding, resulting in a delay of approximately 6 months from the original schedule of research activities at the site. Consequentially, many devices that had already been transported and installed from Japan were temporarily removed to ensure safety. After extensive meetings with regional construction contractors and Malaysian researchers, the concrete base and reinforcement work were re-constructed to reduce the potential of future natural disasters.

(2) Impact of the COVID 19 pandemic
Due to the worldwide coronavirus pandemic that began in early 2020, the governments of Japan and Malaysia have issued a request to restrict all research activities and travel between the two countries, forcing the rescheduling of research activities that were scheduled to take place in FY2020 and resulting in delays in the schedule. The closing of UPM in March 2020 forced the urgent return of Japanese researchers and graduate students who had been staying in Malaysia and the shutdown of all experiments in operation. Therefore, in FY2020, some of the culture experiments that were planned to be conducted in Malaysia were conducted in Japan.

The pandemic continued into FY2021, and research activities in Malaysia were frequently suspended due to the continuous extension of the Malaysian government's restriction order on activities. The original schedule was for two

Japanese researchers to travel to Malaysia from July 2021 to work on the integration of the mass culture system, but in view of the ongoing situation on the Malaysian side, the travel period was postponed until September 2021 or later. In order to achieve mass cultivation of microalgae in Malaysia, a trial run of an outdoor scaled-up multiple bag reactor system was conducted in July 2021 on the campus of Soka University to ensure rapid installation and safe operation of the system in Malaysia. In addition, weekly to bi-weekly meetings were held with researchers in both Japan and Malaysia, as well as with JICA local offices (UPM and KL), making effective use of online tools such as Zoom, to closely coordinate the progress of experiments and field activities and future plans.

4. Lessons Learnt

Through the COSMOS project, we have collectively gained resilience to prepare for and overcome: (i) episodic natural disaster events, (ii) global pandemic restrictions and (iii) collaboration logistics using ICT, learning valuable lessons towards future international collaboration and cooperation.

(1) Research theme 1
(Research group: The University of Tokyo, Leader: Kazutaka Takahashi)

Due to the COVID 19 pandemic, UMT campus activity was banned several times between 2020 and 2021, forcing the suspension of experiments. However, because the microalgae culture strains were under control, the suspended research could be resumed relatively quickly after the university eased its restrictions on research activities.

(2) Research theme 2
(Research group: National Institute for Environmental Studies (NIES), Leader: Akio Imai)

The development and introduction of novel and highly efficient equipment and methods in this study may have led to the achievement of the research objectives, even with many challenges and problems.

Fractionation of humic/non-humic substances using XAD resin, which is required to be carried out under Theme 2, was expected to be very timeconsuming and to require a long time to master the technique. Therefore, we developed a fully automated fractionator that can be used by students and others who are not familiar with experimental operations. In order to promote efficient culture of microalgae under multiple conditions, a culture method utilizing a microplate reader was developed and introduced. These developments and innovations have made it possible to reduce the labor required for culture work and to culture microalgae under a large number of conditions that could never have been achieved by manual flask culture.

(3) Research theme 3
(Research group: Soka University, Leader: Tatsuki Toda)

The outdoor scaled-up multiple bag reactor system introduced and installed in Malaysia in December 2019 was found to be malfunctioning on-site, so after discussing with JICA, the order to the manufacturer and supplier was cancelled under the purchase agreement. We worked at a rapid pace to ensure a speedy installation at the site. A reactor design capable of efficiently agitating the culture media and supplying sufficient sunlight was considered, and the final design was determined after verification experiments. The outdoor large-scale algae culture system was tested in Japan in July 2021, and then transported to UPM in late September of the same year. In particular, because of the severe travel restrictions imposed by the pandemic of the pandemic coronavirus, we received approval from JICA and JST, were included in the travel assignment of government agencies related to JICA, and were issued an entry permit and official passport issued by the Malaysian Immigration Department.

The request was submitted to Soka University for approval and was approved by the president of Soka University as well as by the university's board of directors. Prior to the trip to Japan, we inoculated against the coronavirus (two doses of Moderna vaccine).

(4) Research theme 4
(Research group: Tokyo Institute of Technology, Leader: Kiyohiko Nakasaki)

Because the order to restrict local activities was intermittently enforced, modification and trial operation of the equipment is underway at the demonstration site in time for the end of the order. In addition, the shrimp farms to which the sludge was provided were unable to collect the expected amount of sludge due to multiple outbreaks of shrimp diseases that interrupted their operations, resulting in the cancellation of collection, changes to other farms, and tests using alternative raw materials. Since unexpected circumstances can occur that make it difficult to collect experimental samples as described above, it is advisable to collect and store as large a quantity as possible at one time.

IV. For the Achievement of Overall Goals after the Project Completion

1. Prospects to achieve Overall Goal (ANNEX 6)

After the project ends in FY2022, we will create a management team led by researchers from UPM as a research center, and will continue to promote the "BLUE-SEED" program as Post COSMOS exit strategy (2022 - 2026), and including team leaders from UMT and UNISEL, as well as researchers from four Japanese research institutes.
The overall goal is "**Mass-culture of high value microalgae is adopted by aquaculture farms towards a sustainable aquaculture industry in Malaysia and/or other aquaculture producing countries**". The project aims to develop activities based on four pillars: (1) business and commercialization, (2) research and development (R&D), (3) social engagement and education, and (4) demonstration sites and donated equipment. Details are included in the "3. Tentative Plan after COSMOS Project" of ANNEX 6. The new management (BLUE-SEED) team has already started transition to implementing adoption of the technology to aquaculture farms by securing funding and MOU's. The overall goal will be achieved by continuing the implementation of the exit-strategy plan.

2. Plan of Operation and Implementation Structure of the Malaysian side to achieve Overall Goal

Within 3 to 5 years of the end of the project, we aim to commercialize this microalgae cultivation system for the production of microalgae resources and their use as health food and functional feeding material for cultured fish. Regarding the system for mass cultivation of microalgae using nutrients from sludge, hearings will be held with aquaculture companies and others concerning improvements, set prices, operability, etc., and the commercialization of a low-cost Malaysian-made mass cultivation device will be promoted. Details are included in "3. Tentative Plan after COSMOS Project" of ANNEX 6.

3. Recommendations for the Malaysian side

(1) Among the research institutions on the Malaysian side, UNISEL is a new university established over 20 years ago, and it is necessary to strengthen and

mature the curriculum and research system in the graduate school for capacity development, including research promotion and human resource development.

(2) The elemental technologies and equipment developed in Japan, such as a multi-sequence of operational bag reactors, should be produced locally in Malaysia, and further cost reduction should be realized by local venture companies shown in “2. Key Factors Affecting Implementation and Outcomes”.

(3) The rapid growth in demand for marine resources has led to the release of sludge, the residue from aquaculture, into the ecosystem, which has become an urgent issue. The current situation threatens the food safety of fishery resources due to the spread of the disease. This project will contribute to ecosystem conservation by recovering ammonia, which is a nutrient for microalgae, from sludge as a source of pollution, establishing a new microalgae production process, and creating a recycling process.

4. Monitoring Plan from the end of the Project to Ex-post Evaluation

Details are included in “3. Tentative Plan after COSMOS Project” of ANNEX 6.

ANNEX 1: Results of the Project

- List of the Members from the Japanese side
- Dispatched Experts
- Photo – Members from the Malaysian side
- Allocation of Counterparts
- Photo – Malaysian Members
- Training in Japan and Abroad
- Handover Equipment, Procured Equipment, Certificate
- Renovation/ Rehabilitation of Infrastructure
- Land, Building, Office, and Facilities provided by the Malaysian side
- JICA's Expenditure
- Exchange Rate and Local Execution from August 2016 to March 2021
- MOHE's Expenditure
- Seminar, Meeting and Workshop

ANNEX 2: List of Products

- References
- Manuals

ANNEX 3: PDM (All versions of PDM)

ANNEX 4: R/D, MOA, M/M, Minutes of JCC (copy) (*)

ANNEX 5: Monitoring Sheet (copy) (*)

(Remarks: ANNEX 4 and 5 are internal reference only.)

ANNEX 6: Exit Strategy

Separate Volume: Copy of Products Produced by the Project