R&D area: Polymer Modification
R & D subject No. in project [ 1. Degraded Chitosan for Animal Feed]

Group member: **Bangladesh, Indonesia , Vietnam and Thailand**

**Current status**

Experiment were conducted to investigate the effects of dietary o-chitosan on growth performance in broiler chickens. It has found that feeding of o-chitosan on broiler chicken with regular feed enhanced growth compared to control. (Bangladesh)
The effect of o-chitosan on various animals such as chicken, fish, shrimp and cows has been conducted. The parameter of animals' health in term of blood biomarker and lipid regulation of dairy cattle was evaluated. The finding reveals that o-chitosan could prevent cell damage and also has the ability to regulate lipid homeostasis through good energy management (Indonesia).
The COVID-19 pandemic no longer affects the progress of research projects(Vietnam).In the case of Thailand, the project will be started next year.

**<Remaining/New Challenges>**

Multiple reproducibility should be checked, performance correlation with seasonal diseases, mortality effect (Bangladesh).
Adaptation with the new business process in BRIN in terms of financial support, facilities access, administration support, and networking with external agencies especially the utilization of the R&D product and tech(Indonesia).
The attitude of consumers toward the products prepared by the irradiation method makes these products difficult to apply in daily life (Vietnam)

**Gap in basic aspect:**

Lacking in understanding the effect of molecular weight and functional groups on growth (Bangladesh)

In the case of Indonesia, human resource regarding animal husbandry is limited within Institutes. Research topics in BRIN have changed. The research center should conduct research based on its core technology. If they go to the application, they should connect with the proper institutes that is needs to collaboration.

Vietnam mentioned that little knowledge on mechanism of o-chitosan as an animal feed to increase immune system and growth performance. The inconsistency of o-chitosan molecular weights among countries makes it difficult to compare results.

**Gap in application aspect:**

Knowledge in developing standardized, in-premise poultry facility, product performance under seasonal diseases (Bangladesh)

Need technology takers or industry partners (Indonesia)

It is difficult to register and commercialize research products in agriculture and aquaculture because a new product does not meet standard classification in registration. Limited collaborator between institution and farmer to conduct the field test (Vietnam).

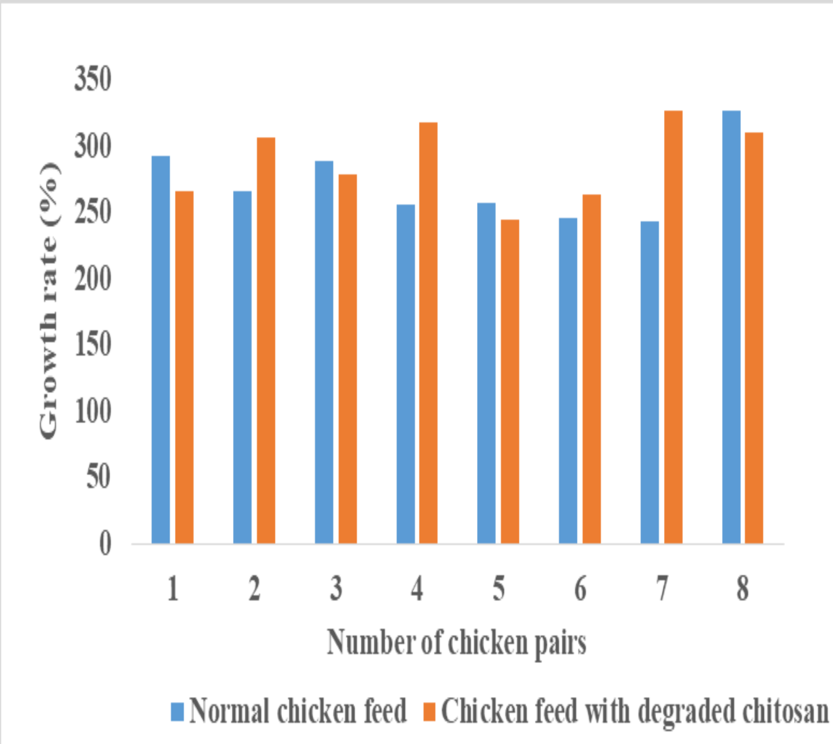
## Implementation plan

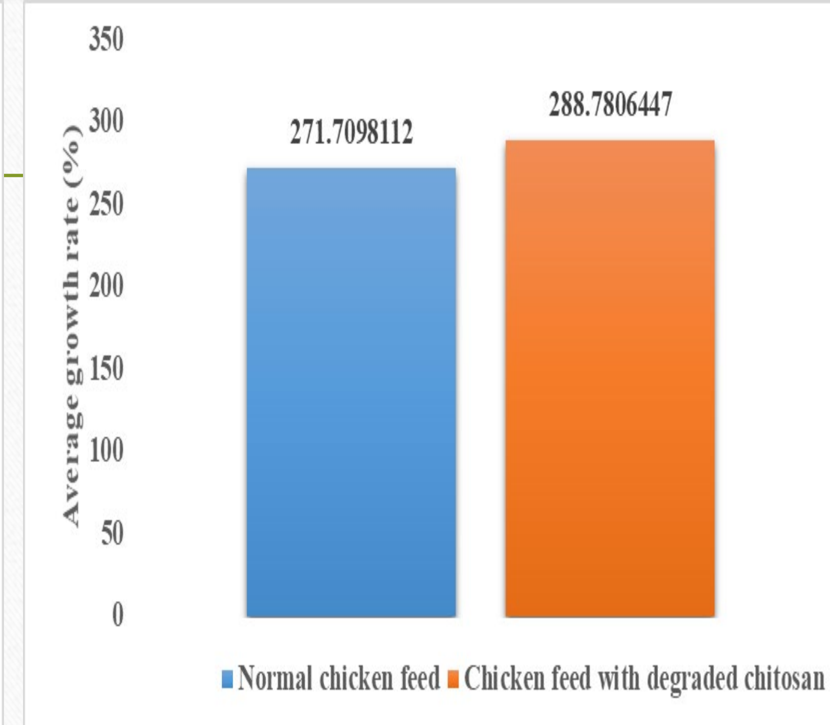
Variation of chicken variety, functionalization of the end product, development of a standardized in-premise poultry facility (Bangladesh).
Make collaboration with the proper Institute to carried out experiment(Indonesia).
Collaboration with other institution which has expertise on animal study and study on the immuno-stimulant and growth performance effect of o-chitosan on another animals such as broiler chicken, pig, cow etc. (Vietnam).

**R&D area: Polymer Modification (Degraded Chitosan for Animal Feed)**

**Group member: Dr. Salma Sultana (Bangladesh)**

**Results of the experiment**




The average growth rate (%) for chicken feed containing degraded chitosan was found higher that was 288. 79 than normal chicken feed that was 271.71. Compared to the control, body weight gain increased in the dietary o-chitosan group 6.29%.

R&D area: Polymer modification

Group member: Dr. Tita Puspitaswari (Indonesia)

Tabel 1. The response of dairy cattle to biomarkers and lipid regulation of heart failure on the low altitude of 300-500 m asl with administration of 500 ppm oligochitosan

| Cardiovascular Biomarkers | Altitude 300 to 500 m a.s.l | |
|---|---|---|
| | Without Oligochitosan | With Oligochitosan |
| CRP High Sensitivity (mg/L) | 27.26±1,44 | 11.83±1,21[a] |
| H-TFABP (ng/mL) | 15.73±1.16 | 6.24±1.03[a] |
| Homocysteine (µmol/L) | 22.51±1.83 | 15.92±2.01[a] |
| γ-Glutamil Transpeptidase (IU) | 36.85±1.64 | 28.51±2.57[a] |
| sPLA2-IIA (ng/dL) | 53,46±2.15 | 46,15±1.01[a] |

Decreasing

| Lipid Regulation | Altitude 300 –500 m asl | |
|---|---|---|
| | Without Oligochitosan | With Oligochitosan |
| Adiponectin ((µg/mL) | 6.67±0.35 | 10.12±0.44[a] |
| Apolipoprotein A-I (g/L) | 1.32±0.54 | 2.55±0.19[a] |
| Apolipoprotein A-II (g/L) | 1.16±0.36 | 2.34±0.89[a] |
| Apolipoprotein B (g/L) | 0.54±0,72 | 1.27±0,08[a] |
| Apolipoprotein C-II (g/L) | 1.27±0.25 | 2.62±0.11[a] |
| Apolipoprotein C-III (g/L) | 1.11±0.25 | 2.07±0.59[a] |
| Apolipoprotein E (g/L) | 1.16±0.25 | 2.53±0.08[a] |
| Cholesterol HDL (mg/dL) | 43.63±2.12 | 79.35±3.04[a] |
| Cholesterol LDL (mg/dL) | 76.16±3.62 | 99.05±4.06[a] |
| Cholesterol total (mg/dL) | 135.27±3.78 | 188.19±5.02[a] |
| Triglycerides (mg/dL) | 152.52±3.21 | 282.74±5.07[a] |
| NEFA (mg/dL) | 46.42±2.33 | 69.81±4.06[a] |

Increasing

Thank you all

R&D area: 2. Hydrogel for Medical Application (2022)

Group member: Charito T. ARANILLA (Philippine), Mitsumasa TAGUCHI (Japan)

Current status

<Improvements from 2019>

Philippines:

- Developed hydrogel hemostat prototypes through radiation crosslinking
- Completed biocompatibility, shelf-life and efficacy in animal bleeding models
- New approved projects for Clinical Trials and Upscaling Production

Japan:

- New 3D cell culture materials were developed to investigate and control cell responses from natural polymers for advanced medicine by using radiation crosslinking.
- Nano-particle type sensors were developed for safer MRI diagnostics by using radiation technique.

<Remaining/New Challenges>

Philippines:

- Establish radiation processing of hemostat using E-beam technology
- Preparation of full clinical trial protocol
- Approval of clinical trial protocol from Institutional Ethics Regulatory Board

Japan:

- In vitro and in vivo tests for medical applications.
- An up-scaling production method of hydrogels and nanogels will be developed in order to apply in the biological/medical fields.

R&D area: 2. Hydrogel for Medical Application (2022)

Group member: Charito T. ARANILLA (Philippine), Mitsumasa TAGUCHI (Japan)

<u>Gap in basic aspect</u>

Difficult to conduct experiments and have meetings due to COVID-19 (Japan and Philippines)
Delays in delivery of purchases and out of stock goods (Japan and Philippines)

<u>Gap in application aspect</u>

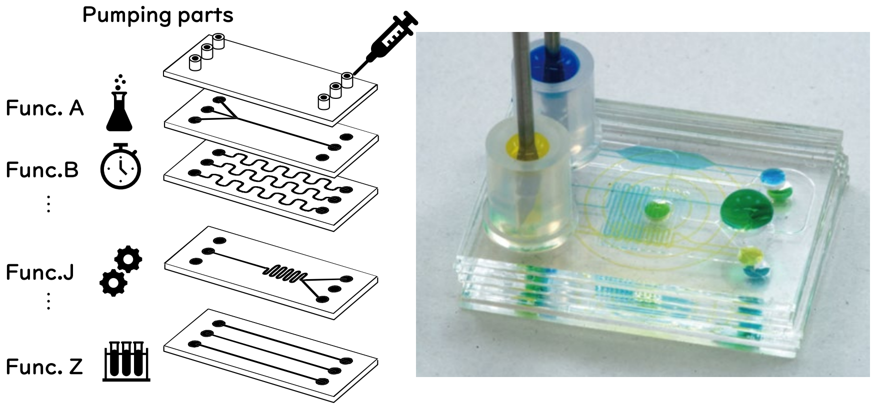
Limited irradiation facility (Philippines)
Need for GMP facility with License for Biomedical Device Production for upscaling (Philippines)
Properties of products need to conform to FDA standards (Philippines)
Need technology takers or industry partners (Japan)

<u>Implementation plan</u>
Hydrogel hemostats in commercialize-able form (Philippines)
Initiate Technology Transfer Process (Philippines)
Hydrogels for regenerative medicine, and drug discovery (Japan)
Nanoparticles and microfluidics for diagnostics (Japan)

R&D area: 2. Hydrogel for Medical Application (2022)

Group member: Mitsumasa TAGUCHI (Japan)

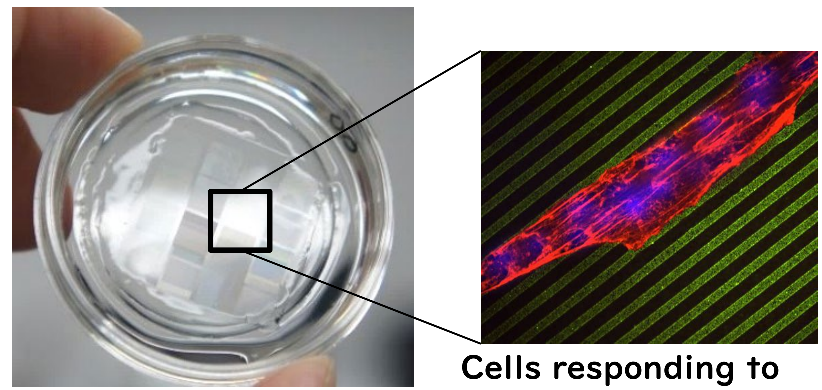
## 1. Multiply stacked microfluidic chips




**Analytical performance increased**

T. G. Oyama, *Lab. Chip, 2020*

## 2. 3D culture hydrogel substrates

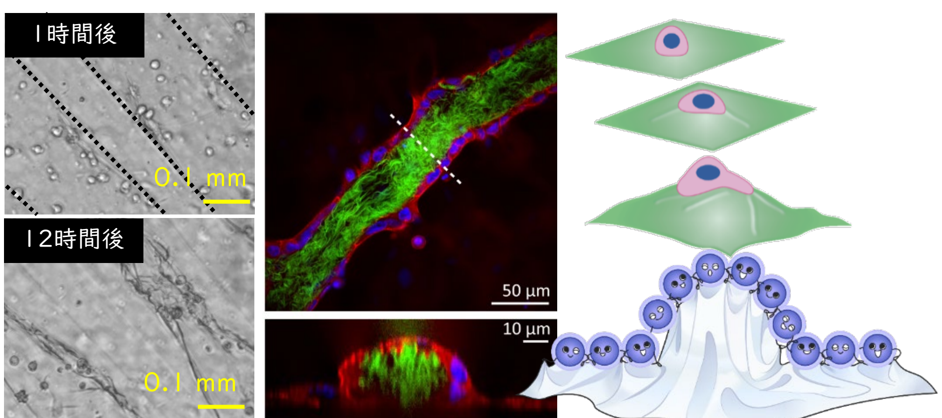


Cells responding to stiffness and shape.

**Microfabrication of collagen gels**

T. G. Oyama, *Biomedical Materials,* 2021

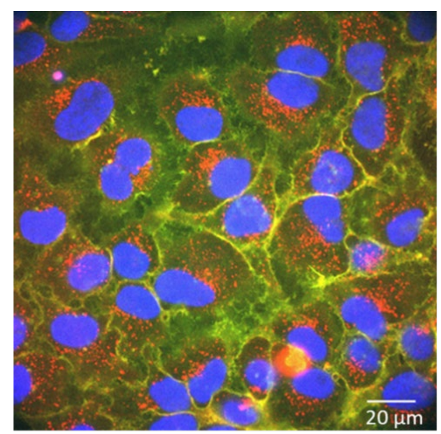
## 3. 3D cell sheets




**Cells deform films to protrusions**

T. G. Oyama, *Materials and Design*, 2021

## 4. Nanosensors



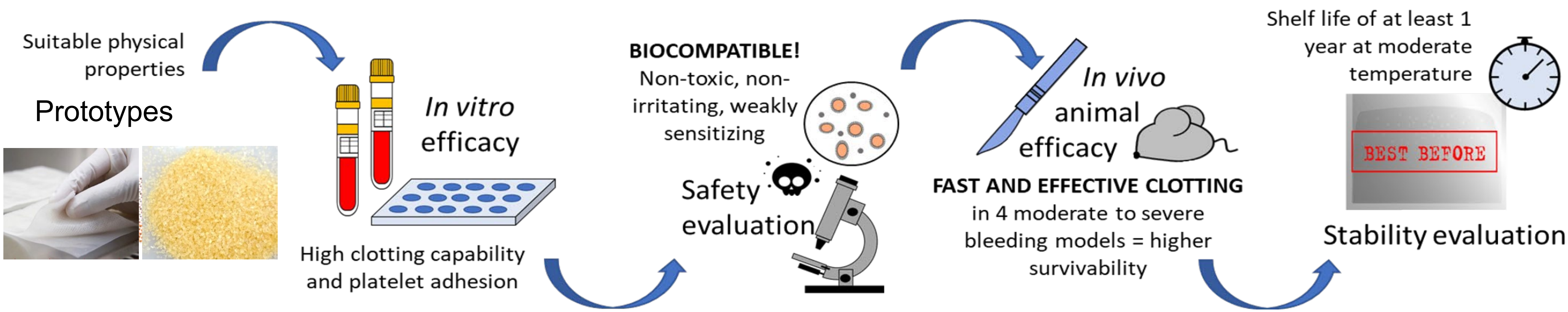

**Accumulated in cancer cell**

A. Kimura, *Nanomaterials*, 2021

# R&D area: 2. Hydrogel for Medical Application (2022)

## Group member: Charito ARANILLA (Philippines)

**Completed:** Prototyping, Biocompatibility, Shelf-life and Animal Studies




**New Project for 2023:** Clinical Trial and Upscaling Production of Hydrogel Hemostats

**Framework**

**Prototype Hemostatic Devices (TRL 4/5)**
(1) Carboxymethyl Cellulose Granules
(2) Kappa-carrageenan/Polyethylene oxide/ Polyethylene Glycol Dressing

**Multi-center Clinical Trials**
Safety and efficacy in the management of trauma wound bleeding in prehospital and hospital emergency settings.

**Upscale GMP Production Trials**
(1) GMP process development
(2) Optimization of radiation processing parameters
(3) Validation and substantiation of 25 kGy as radiation sterilization dose
(4) Product Shelf-life

**Cost-effectiveness Analysis (CEA)**

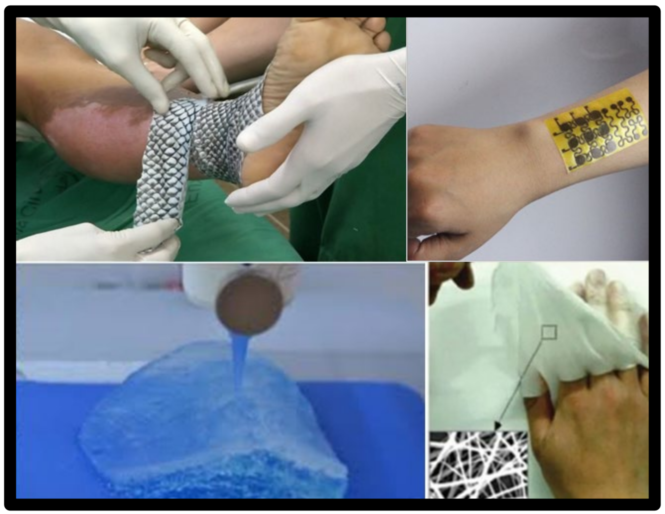
**Hemostatic Devices (TRL 9)**
(1) Technology Transfer
(2) FDA Registration
(3) Commercialization

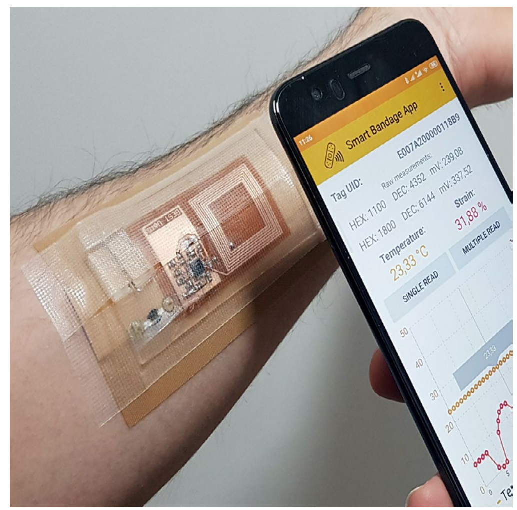
**Stakeholders**

R&D area: Special topics cited from presented PPT (2022)

Group member: Charito ARANILLA (Philippines)

**S**mart **M**ultifunctional Hydrogel **D**ressings sterilized under an **E**lectron beam as novel wound **R**epair **M**atrices






**Integrate pH and moisture sensors and Develop smart phone app**

R&D area: 4. Synergistic Effect among Plant Growth Promoters, Super Water Absorbents and Biofertilizer

Group member: Dr. Fathoni (Indonesia), Prof.Tawaraya (Japan), Dr. Nagasawa (Japan), Ms. Mahmud (Malaysia), Ms. Otogonbayar (Mongolia), Ms. Anarna (The Philippines), and Dr. Prongjunthuek (Thailand)

## Current status

Improvements from 2019:

- In this topic all of the member country reported different **biofertilizer** products, plant growth promoters and super water absorbents with significant effect on plant growth, improved soil health, increased crop yield and reduction in the utilization of chemical fertilizer up to 50%. Continuous experiments and evaluation were conducted to evaluate the effect of biofertilizer in different crops and the synergy effect of combined inoculation with different microbes and chemical inputs whether fungicide or chemical fertilizers. Some of the products coming from Thailand, Philippines, Malaysia, Indonesia and Japan are already licensed/commercialized and were already transferred to private sector. Biofertilizer produced is now being accepted by the farmers and others are being supported by the government.

**Remaining/New Challenges:**

Based on different reports of each country identification of different microorganism will be conducted, analyzed and tested to different crops as biocontrol or biofertilizer as reported by Dr Okasaki Shin of Japan to identify the mechanism of the microbes. Others will continue synergetic study on optimum ratio dosage of Tebutin (fungicide) and biofertilizer (Mongolia). Enhancement of the production techniques to make the product readily available. Quality assurance/control of the product to determine the survival of microorganisms to assure that the product has good quality (Indonesia). New methods for preparation of concentrated microbial inoculum and preservation. (*Azotobacter vinelandii* and *Burkholderia vietnamiensis* Marketing and commercialization of the technology (Thailand and Philippines). In Malaysia , to date Nuclear Malaysia has commercialized four BF products. By application o - f BF, farmers are able to reduce chemical fertilizers usage, increase yield 8-12% and 20-35% income. In future, application of BF and nutrient uptake analysis using drones will enhance efficiency and effectiveness of BF application in modern agriculture.

**Gap in basic aspect**

1. Availability of supplies and materials
2. In adequate  labor force
3. Lack of awareness on gamma irradiation technology utilization

**Gap in application aspect**

1. No available cooperators for field test
2. Climate change
3. Technology transfer from lab to Industry is still challenging
4. Difficulties in looking for stakeholders

**Implementation plan**

Continuity of the Research and Development on the following aspect

1. Improved microbial strains

2. Metagenomic, meta-transcriptomic, and metabolomic study of the identified microbes

3. Search for the suitable carrier for different type of biofertilizer (a consortium of bacteria, fungi, *etc.*),

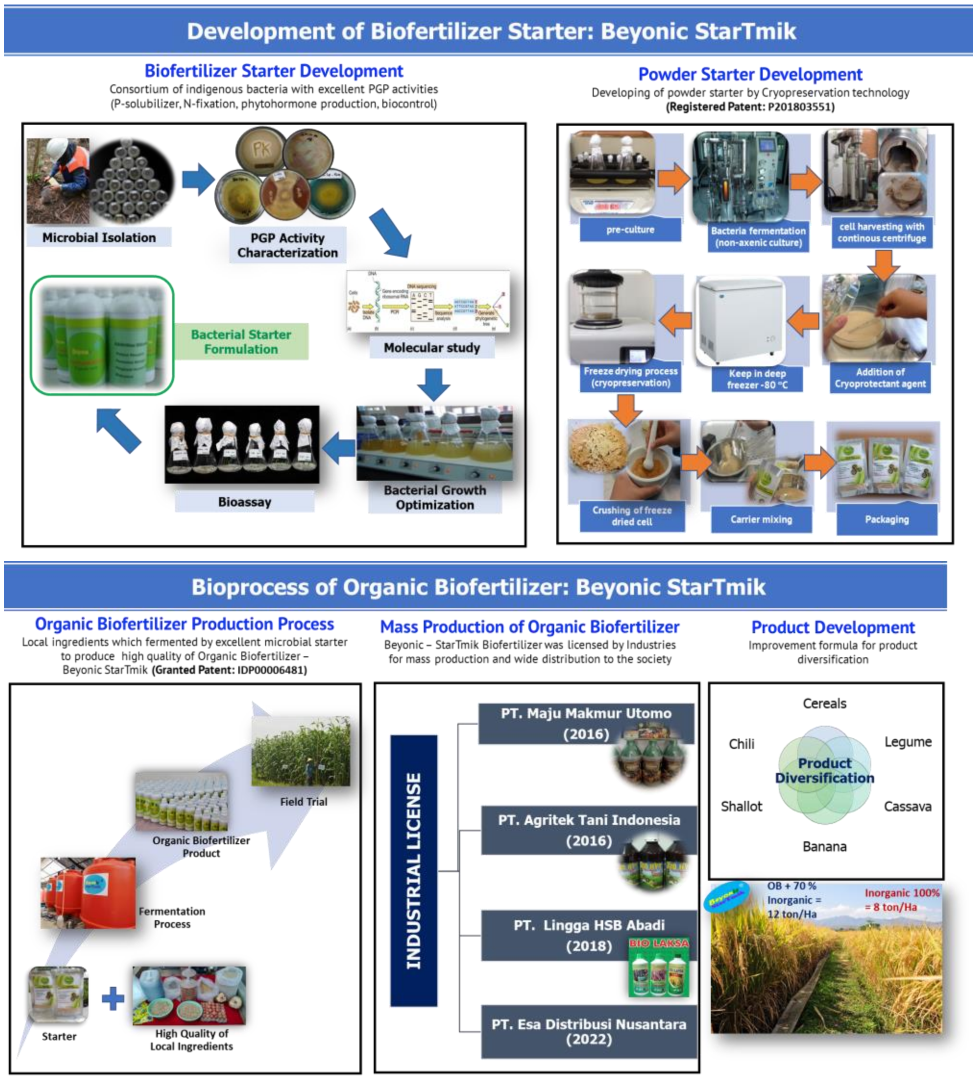
4. Optimization of the production on the large scale

5. Improvement of equipments/techniques for biofertilizer production.

6. Promotion and Extension of PGPB, SWA and Biofertilizer

# Indonesia

## Organic Biofertilizer for various crops and vegetables




BRIN
BADAN RISET DAN INOVASI NASIONAL

*Beyonic StarTmik*

**Licensed by four companies:**

1. PT. Maju Makmur Utomo,
2. PT. Agritek Tani Indonesia,
3. PT. Lingga HSB Abadi,
4. PT. Esa Distribusi Nusantara.

**Future planning**:

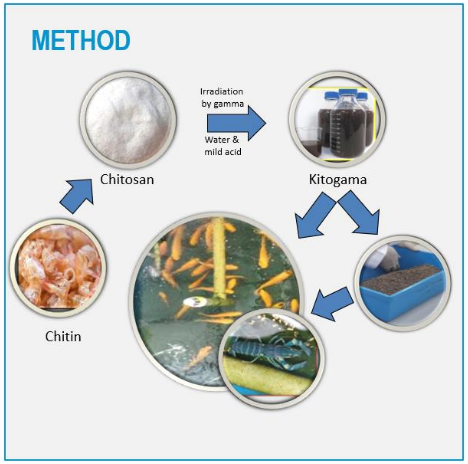
- Diversification of products for specific commodities.
- Strain improvement using mutation radiation

**Processed and ongoing:**

Cassava
Shallot,
Banana,
Cereals,
Legumes
etc.

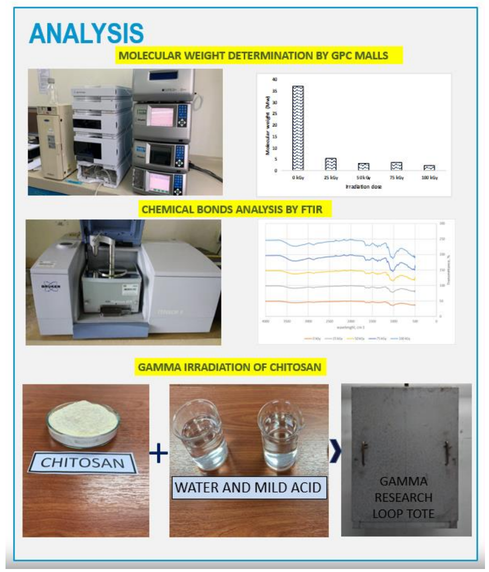
# Malaysia

## DEGRADAED CHITOSAN FOR ANIMAL FEED

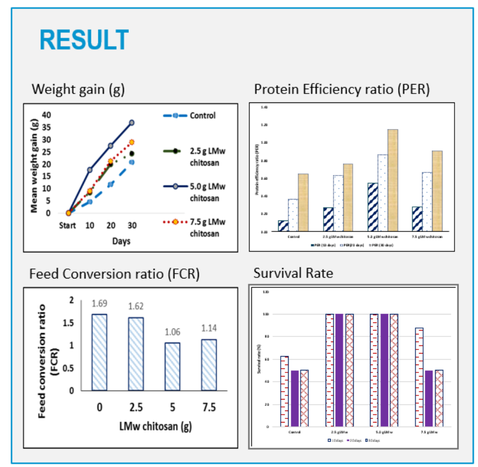



1. Preparation of Kitogama (degraded chitosan for animal feed) is established.


3. We identified method of application of Kitogama, by (i) pour into the water tank, (ii) add in the feed (fish feed and silage)



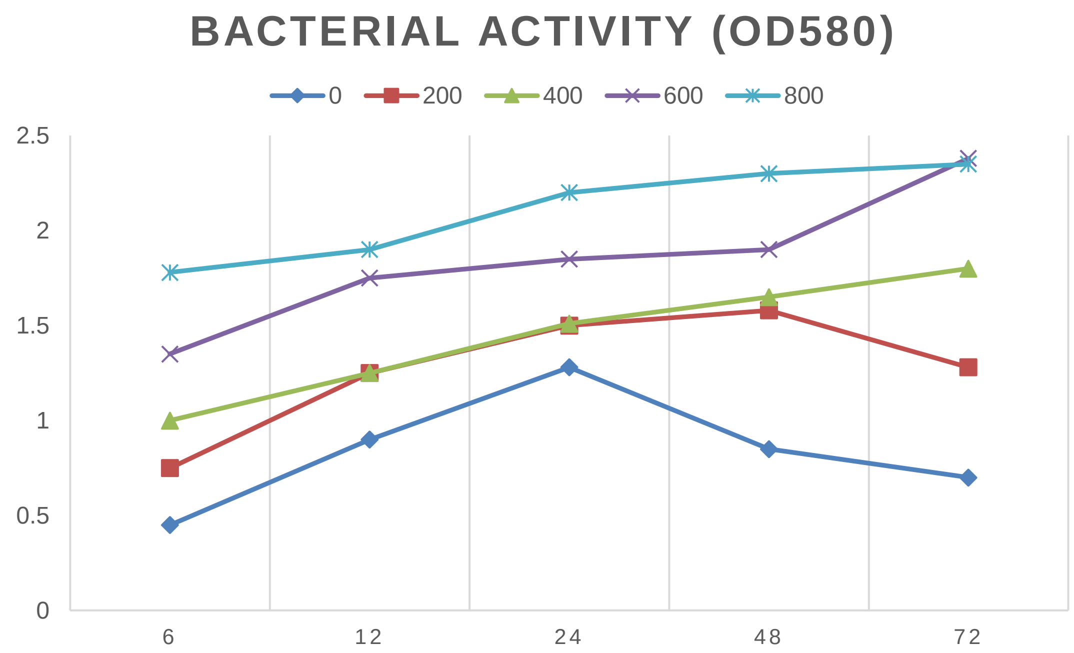

2. Quality assessment/control for every batch of raw materials and product to produce quality Kitogama.



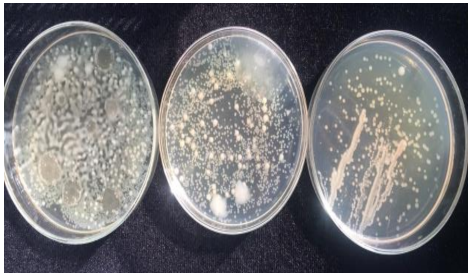

4. Study the effect of Kitogama on tilapia (2020), lobster (2021), chicken (2021) and cow (2018).

Mongolia

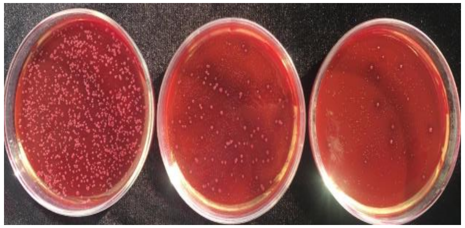
## Combination of Tebutin (fungicide) and biofertilizer




** Determination used the spectrophotometer (at 580 nm)*



Azospirillum&azoarcus on NA



Bacteria on NA with tebutin of maximum dose

- Tebutin (fungicide) as seed fertilizer has no significant impact on bacterial activity.
- In average yield of 2021-2022, bacterial fertilizing plots were 6.9-9.0 centner/ha higher than control.

**Philippines**

## Production and Utilization of Bio-organic Fertilizers Enhanced with Biofertilizers (*Bio N* and Mykovam) for Organically Grown Crops

This project aims to produce and utilize the enhanced bio-organic fertilizers for organically grown crops with emphasis on approaches that will promote organic farming.
Result: Formulation of enhanced bio-organic fertilizer through addition of microbial inoculant (nitrogen fixing bacteria and mycorrhiza)

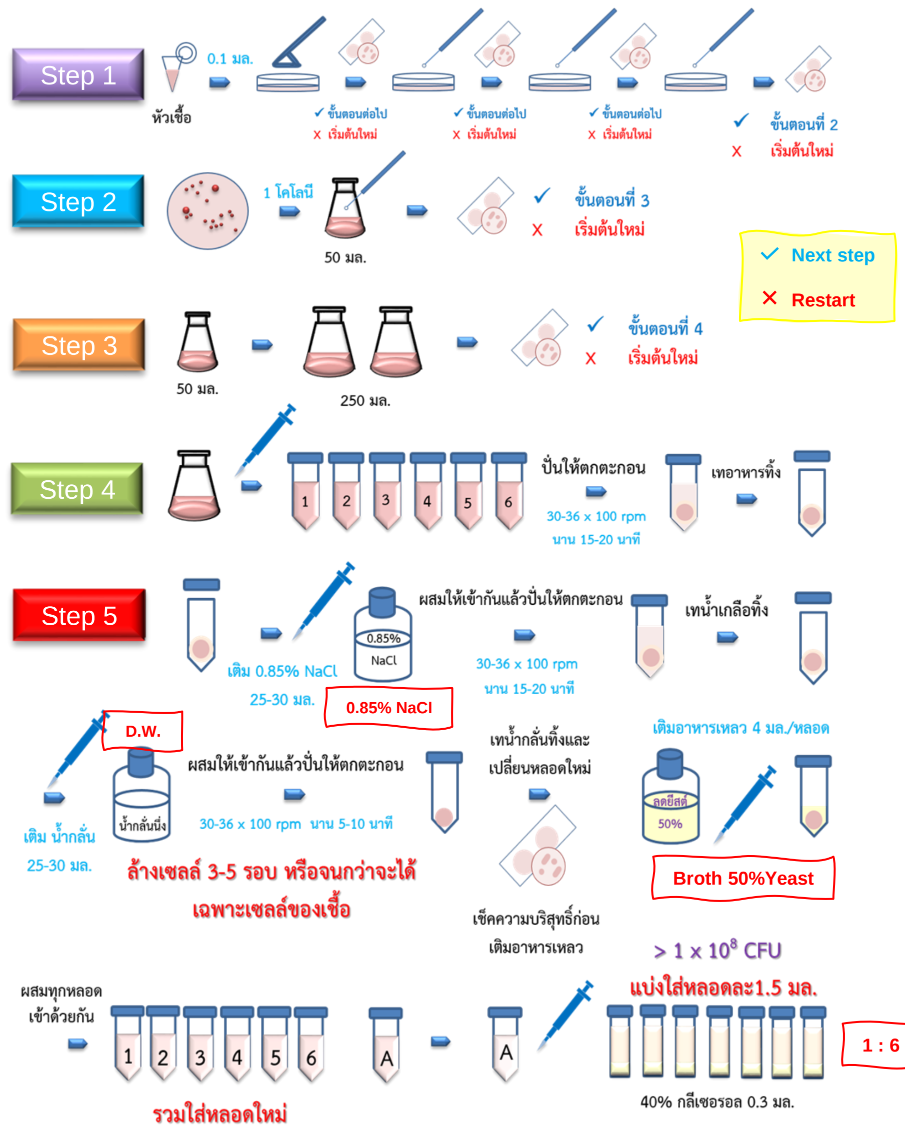
The data also revealed that farmer can harvest enough even without application of chemical fertilizer with the right amount of bio-organic fertilizers

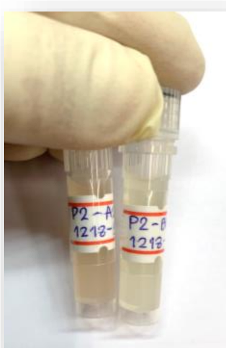
| Treatment | Total number of fruits | Percent Inc. Over control | Weight (kg) of fruits | Percent Inc. Over control |
|---|---|---|---|---|
| T1 - Bio-organic fertilizers | 635 | | 36.55 | |
| T2 - Enhanced Bio-organic Fertilizers | 922 | 45.20 | 47.85 | 30.91 |
| T3 - 50% Enhanced Bio-organic fertilizers | 916 | 44.25 | 42.75 | 17 |

# Thailand

PGPR-I in cannabis cultivation was found to help cannabis grow and flowering faster and have wax coating on the leaf surface.

New methods for preparation of concentrated microbial inoculum and preservation.



*Azotobacter valendii and*

*Burkholderia vietnamiensis*

- Clean with D.W. 3 times
- Glycerol and inoculum ratio 1:5
- Keep 4ºC

## R&D area: Group D: 6. Mutation Breeding of Microbe Using Radiation (2022)

Group member: Dr. Chinzorig RADNAABAZAR (Mongolia),
Dr. SATO Katsuya (Japan)
Dr. TRAN Minh Quynh (Vietnam)
Dr. PHUA CHOO Kwai Hoe (Malaysia)
Dr. DAS Pronabananda (Bangladesh)
Dr. HASE Yoshihiro (Japan)

Current status

(Malaysia) Gamma ray (doses of 100 to 500 Gy) was applied to *Acinetobacter* sp to induce mutants. One mutant was .4 Phosphate solubilizing (pqq) genes were isolated from M100 mutant.

(Japan) Characterization of mutations by ion beam & gamma rays irradiation using *Bacillus subtilis* spore.
Ion beam might more induced large sized mutation than that of gamma rays.

(Vietnam) Two Trichoderma (T.koningiopsis VTCC 31435 and T.reesei VTCC31572) strains with high and stable production of cellulase, were selected and irradiated by gamma ray within the dose of 300 to 2500 Gy. The dose range of 700 to 1500 Gy produced broad frequency of mutants. After screening, five potential mutants were selected, and one mutant in genome (insertion at 413 and substitution at 325 positions) was confirmed by sequencing.

## Remaining/New Challenges

Starting material, type of mutation source, doses, number of screening generation, stability of the mutant strains, methods.

Analysis of relationship between large size mutation and phenotype.

## Gap in basic aspect

High cost (especially for whole genome sequencing)

Standard protocol for mutagenesis

## Gap in application aspect

Concerns about mutants become antagonist/ pathogen etc.

Regulation of application (Malaysia and Mongolia)

## Implementation plan

Looking for financial supports for further studies

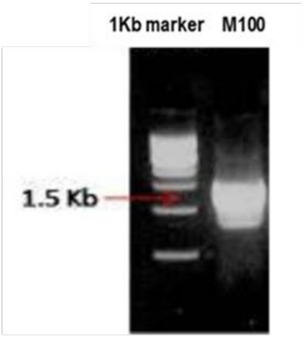
Large scale application of the obtained mutants

Guideline for mutagenesis

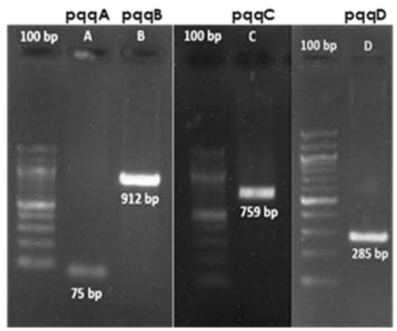
## Malaysia

**Title:** Investigation of mutagenesis of phosphate solubilizing microbe for biofertilizer using gamma irradiation

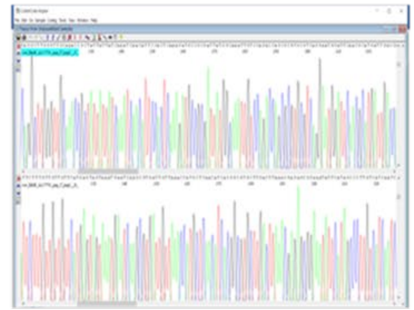
**Summary: Four *pqq* genes (A, B, C, D) of mutant M100 has been isolated.**



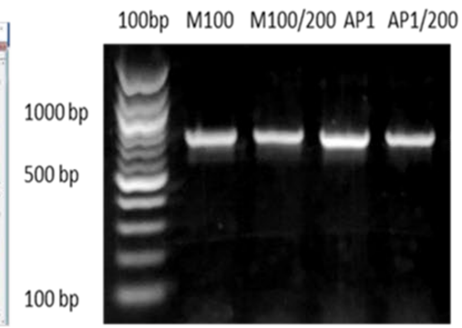

Biofertilizer bacteria genome extraction




pqq genes isolation



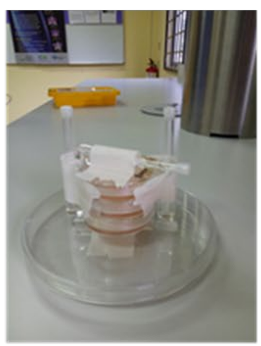
Pqq genes sequences

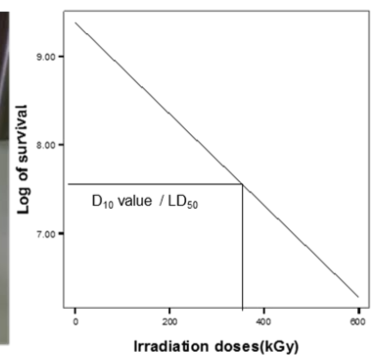



Biofertilizer bacteria mutagenesis study (Irradiated/ non-irradiated)

**Title:** Developing standard guideline for Gram positive and negative bacteria mutagenesis by using gamma cell

**Summary: Mutagenesis and determination of $LD_{50}$ of bacteria was done.**






**Gamma irradiation for mutagenesis biofertilizer bacteria guideline**

**Table of Contents**



Development of guidelines on mutagenesis of Gram positive and negative bacteria.

# Japan

**Fungi**

*Aspergillus oryzae*
*Aspergillus sojae*
*Aspergillus nigar*
*Trichoderma reesei*
*Rhizomucor miehei*
*Isaria fumosorosea*
*Beauveria bassiana*
*Metarhizium anisopliae*

**Yeast**

*Zygosaccharomyces rouxii*
*Saccharomyces cervisiae*
*Alcohol fermentation yeast*

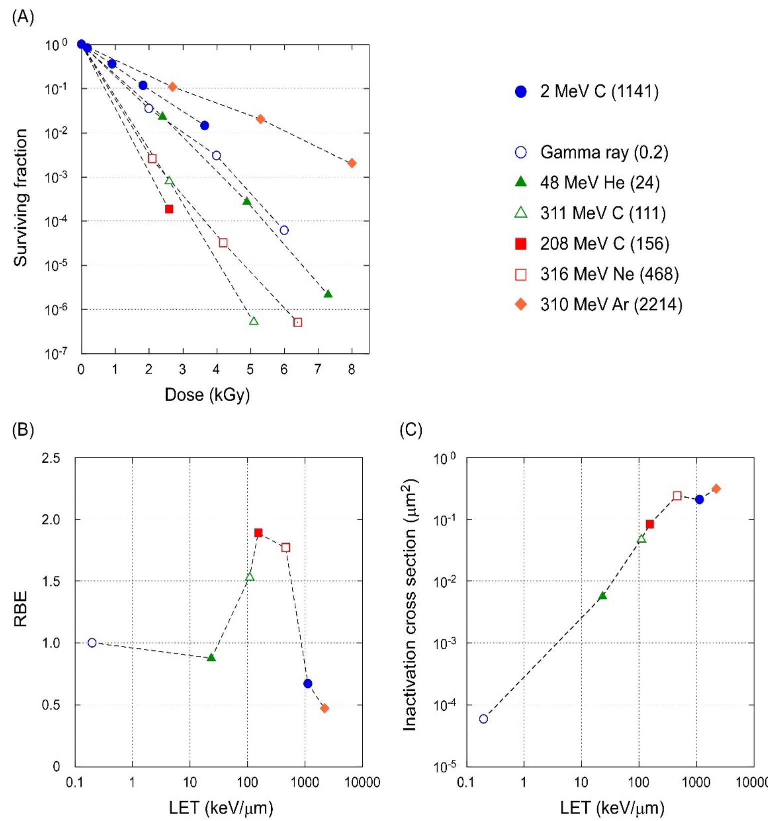
**Microalgae**

*Tisochrysis lutea*
*Chlamydomonas* sp.

**Bacteria**

*Bradyrhizobium japonicum*
*Bacillus subtilis*
*Deinococcus radiodurans*
*Rhodococcus erythropolis*

Enzyme production
Biopesticides
Fermented foods
Biofuel
Biofertilizers
Environmental remediation
…*etc*

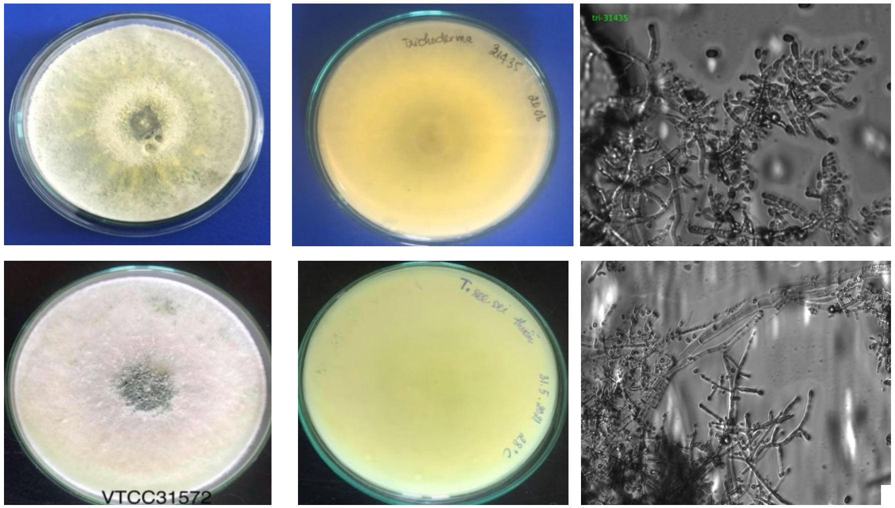



**Breeding of microorganisms using ion beam breeding technology at QST-Takasaki.**
The ion beam breeding technology at QST-Takasaki has been applied to the development of industrial microorganisms.

**Effect of LET on the lethal effect, RBE, and inactivation cross section in *Bacillus subtilis* spores.**
(A) Dose response for surviving fraction.
(B) LET-RBE relationship based on $D_{10}$.
(C) Inactivation cross section as a function of LET. Values in parentheses represent the LET (keV/μm) for each ion species.

Vietnam

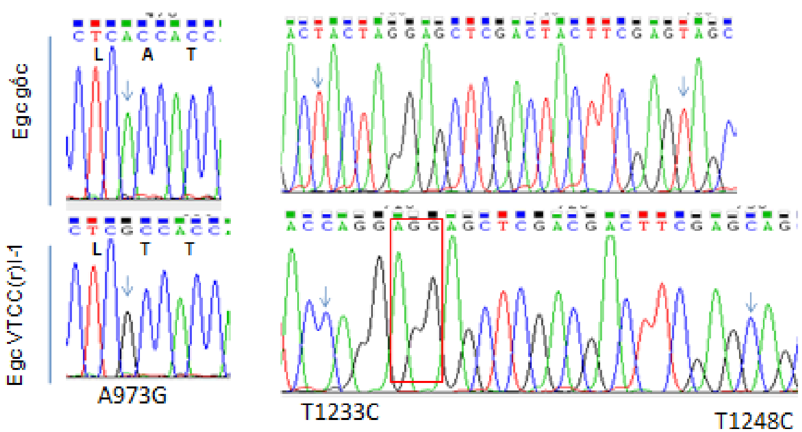
## 1. Selecting initial *Trichoderma* strains




## 2. Screen potential mutant strains with higher cellulase production

| No. | Mutant colonies | HC value |
|---|---|---|
| 1 | *T. reesei* VTCC 31572 | 1.87 |
| 2 | VTCC(r) I-1 | 2.71$^c$±0.06 |
| 3 | VTTC(r) I-2 | 2.25$^a$±0.05 |
| 4 | VTTC(r) I-3 | 2.15$^a$±0.11 |
| 5 | VTCC(r) I-4 | 2.17$^a$±0.02 |
| 6 | VTCC(r) I-5 | 2.45$^b$±0.05 |

## 3. Decoding and analyses of the differences in Endonglucanase coding genes of radiation induced mutants of *Trichoderma*




**Changes** in nucleotide lead to the changes of amino acid in the endoglucanase gene of VTCC(r) I-1 compared to the gene of wild strain (**Arrow** showed the changed nucleotide; **Rectangular** showed the insertion code)

## 4. Optimizing the conditions for solid state fermentation of mutants

**Optimal parameters for the solid state fermentation of radiation induced mutants**

| Parameters for SSF | Optimal conditions | |
|---|---|---|
| | VTCC(k) I-1 | VTCC(r) I-1 |
| Medium pH | 6.5 | 6.5 |
| Incubation temperature (°C) | 30 | 33 |
| Fermentation time (days) | 7 | 7 |
| Seed / substrate (%) | 10 | 10 |
| Solid substrate for fermentation | Rice bran/husk/molases (6.0 - 3.5 - 0.5) | Rice bran/husk/molases (6.0 - 3.5 - 0.5) |
| Moisture (%) | 60 | 60 |
| Cell density in seed (CFU/g) | 14.38x $10^9$ | 17.93x$10^9$ |

R&D area: 3. Environmental Remediation (2022)

Group member: Dr. Duy (Vietnam), Dr Sultana (Bangladesh), Dr. Fathoni (Indonesia), Dr. Sato and Dr. Taguchi (Japan)

Current status

<Improvements from 2019> (specify the name of the country if needed)

- Improvement of microbial immobilization for bioremediation. Application in several batik and textile industries has been conducted. Application of indigenous AMF isolates for post-mining land reclamation is on going with industry (Indonesia)

- Analysis of the cesium (Cs)-accumulating mechanism in the radioresistant bacteria. (Japan)
- 
- Treatment of azo dye residues from textile wastewater by electron beam irradiation combine with biological treatment in large scale. (Vietnam)

R&D area: Environmental Remediation (2022)

Group member: Dr. Duy (Vietnam), Dr Sultana (Bangladesh), Dr. Fathoni (Indonesia), Dr. Sato and Dr. Taguchi (Japan)

Current status

<Remaining/New Challenges>

- We are inspired after utilizing both the irradiated and non-irradiated PET waste-flakes within concrete blocks after the preliminary observation of the tensile strength being almost similar to the control (without PET flakes). (Bangladesh)

- Transferring technology to industry. Improving microbial strain using radiation mutation. Finding low cost microbial immobilization for bioremediation. Finding new microbial isolate to degrade wax component in batik/dye wastewater. Using mycorrhizal-seed balls and mycorrhizal-bio-pots in the revegetation with cover crop for reclamation of post-mining land. (Indonesia)

- Development of some useful microorganisms through the mutation breeding technology for bioremediation.(Japan)

- Pollutant dioxin in the soil, pesticides and organic pollutants from wastewater hospital is big challenges (Vietnam).

R&D area: Environmental Remediation (2022)

Group member: Dr. Duy (Vietnam), Dr Sultana (Bangladesh), Dr. Fathoni (Indonesia), Dr. Sato and Dr. Taguchi (Japan)

Gap in basic aspect

- Optimization in the component materials, and radiation dose for highest strength and minimum brittleness
- Research is still lacking in utilization of mutant/recombinant microbes on bioremediation. Research is still lacking in the field of metagenomic in the contaminated area (Indonesia).
- Microbial activity in bioremediation is less stable. (Japan)
- When treated with sunlight, the efficiency of photocatalysts made of Ag nanoparticles/$TiO_2$ remains low (Vietnam)

Gap in application aspect

- For scaling up to pilot scale, mixing or grinding machines are needed to achieve a homogenous mixture of the component materials (e.g. PET flakes, gravel, sand, cement and water). (Bangladesh)
- There are limited study on the application of microbe and enzyme in bioremediation process in the WWTP. Scale up production for field application (Indonesia)
- Assessment of ecological effects and human health effects by bioremediation. Collaboration with many organizations for expansion of bioremediation (Japan)
- Lack of irradiation facilities for application development to treat wastewater (Vietnam)

R&D area: Environmental Remediation (2022)

Group member: Dr. Duy (Vietnam), Dr Sultana (Bangladesh), Dr. Fathoni (Indonesia), Dr. Sato and Dr. Taguchi (Japan)

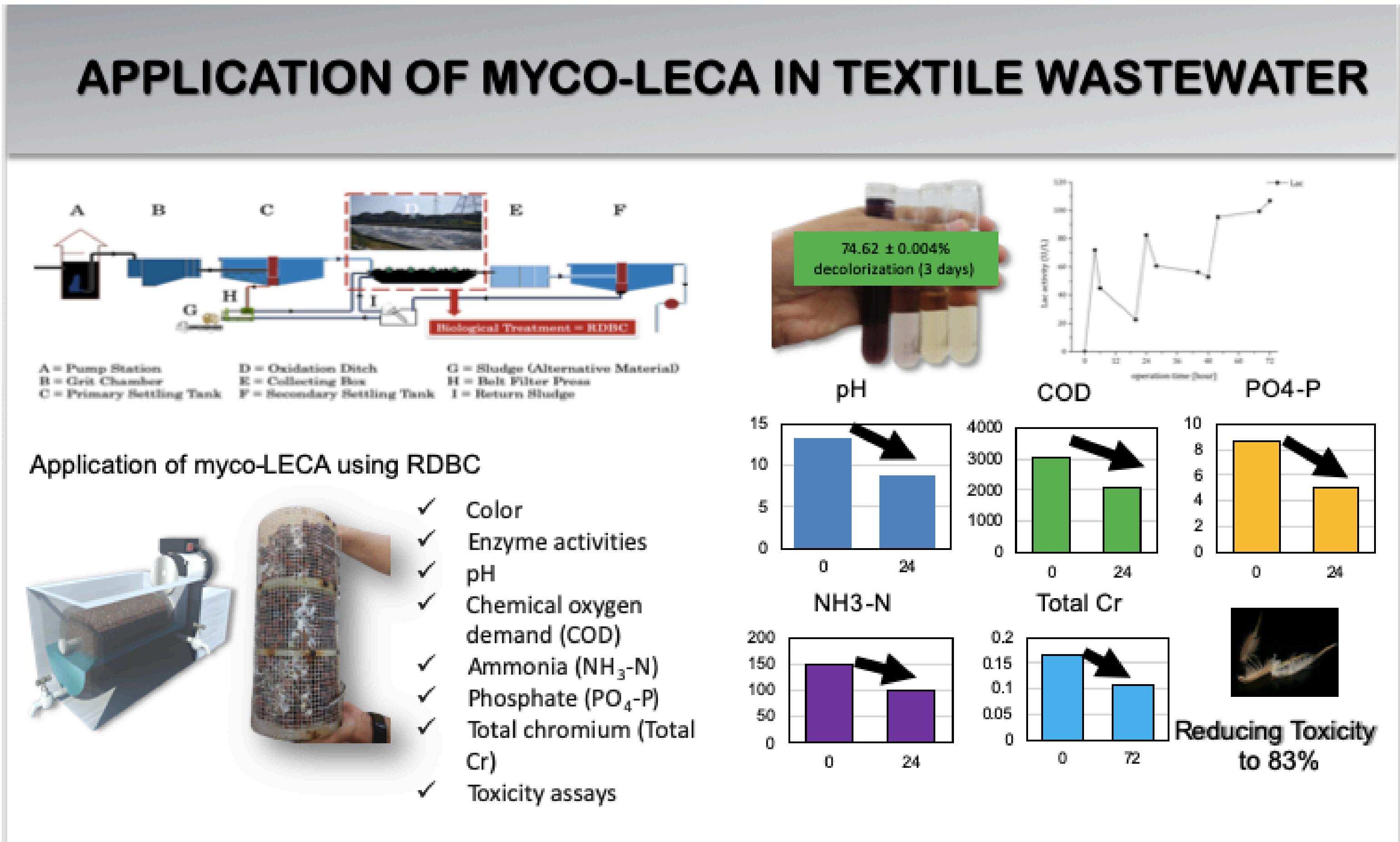
Implementation plan

- Based on industrial consultation, we want to optimize of the cement-to-PET ratio, the binding materials and the radiation dose and reduce the amount of sand for the maximum strength and minimum brittleness of the concrete-blocks. (Bangladesh).
- Improve collaboration with local government and industry to implement the technology. Finding suitable method for mass production of bioremediating agent. Monitoring and continuing assessment of the polluted environment before and after bioremediation. (Indonesia).
- Study on the elucidating of the Cs-accumulating mechanisms in useful microorganisms by the comparative genome analysis. (Japan)
- Study on the treatment of dioxin, pesticides and organic pollutants from wastewater hospital by electron beam method.. Synthesis of Cu nanoparticles/$TiO_2$ and Cu-Ag nanoparticles/$TiO_2$ by electron beam irradiation for photodegradation of organic pollutants in water (Vietnam).

R&D area: 3. Environmental Remediation (2022)

Group member: Ahmad Fathoni (Indonesia),

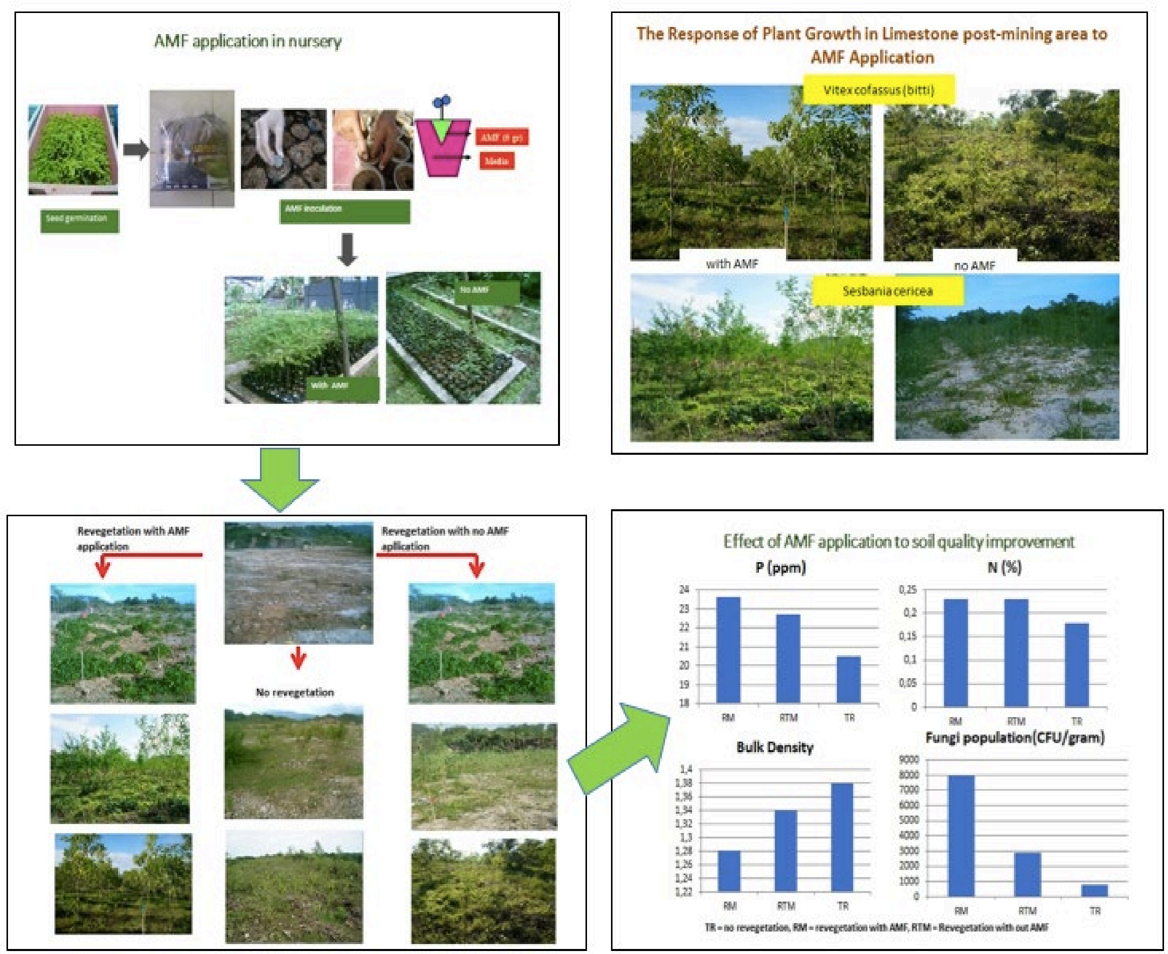
## Special topics



R&D area: 3. Environmental Remediation (2022)

Group member: Ahmad Fathoni (Indonesia),

## Special topics



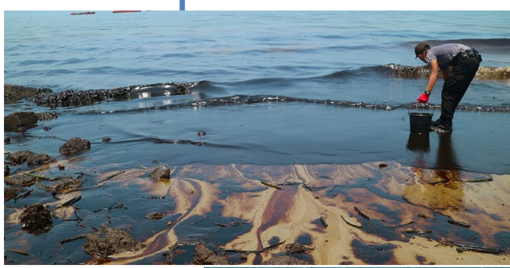

Indigenous AMF isolates for post-mining land reclamation

| 2 | **Biocarrier formulation of POP-degrading microorganisms (Crude oil and Microplastic) and identification of degradation products** |
|---|---|

Crude oil and microplastic contamination survey and Biocarrier-technology

**2022**

Survey, sampling, mapping and data collection

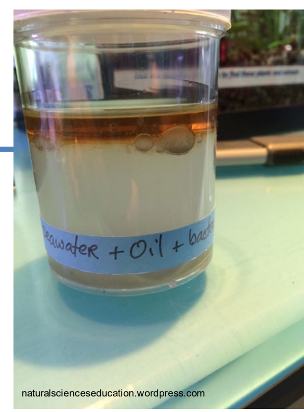



**2023**

Metagenomic analyses, laboratory & mesocosm study

Biocarrier formulation

(Gamma irradiation)




**2024**

Field trial (in situ treatment of crude oil and microplastic contamination)

R&D area: Environmental Remediation (2022)

Group member: **Dr. Salma Sultana** (Bangladesh),

## Preparation of γ-irradiated PET-concrete blocks

**Within a Mould**

Sand : Cement : Water : **PET (irr./non-irr.)**

92 : 46 : 47 : **0.5**

Curing at R. T.

for 28 days


| Sample | Tensile strength (Mpa) |
|---|---|
| **Control** | 8.60 |
| **Non-irradiated PET** | 8.34 |
| **Irradiated PET (50 kGy)** | 9.09 |

**PGP and SWA, inclusive process development**
**Malaysia (Maznah Mahmud), Mongolia (Ms Sunjee Otogonbayar) and Philipines ( Dr. Charito T. Aranila)**
Achievements

Malaysia
1. Registration for Copyright in Malaysia No. CRLY2022W02283 for “Procedures for Preparation of Nuclear Malaysia Radiation-Degraded Chitosan (NM-Oligochitosan) as Plant Growth Promoter”
2. Procedures on preparation of CarraPGP using gamma ray is established.
3. Procedure of development Sago waste-based SWA using gamma ray is established.
4. CarraPGP cheaper and the processing time is shorter to NM Oligochitosan

Mongolia
1. The liquid Rhizobacterial fertilizer increases yield of wheat grain by 25-33%, seed potato yield by 42.3-45.6% and yield of vegetable crops /carrot, onion, tomato, sweet pepper et.c/ by 22-50 %
2. In 2019-2020, showed synergy effect of liquid bacterial fertilizer and Oligochitosan increase crops yield by 50-80%. Biofertilizer and oligochitosan are significantly effective for plant growth promotion.
3. In 2021, synergistic effect of liquid bacterial fertilizer and Oligochitosan increased potato yield by 46-50% and about 4.1-8.9 t/ha more than control

Philippines:
1. Developed PAA/Starch SWA with optimum formulation
2. Studied biodegradability, conducted safety evaluation and determined stability
3. Translated production from laboratory to pilot scale
4. Proven efficacy in pot and field tests
5. Performed cost-benefit analysis
6. Studied effect of retrogradation and its effect on gel stability
7. Project ended in in June 2022
8. Published 1 paper

**PGP and SWA, inclusive process development**

Current status

Malaysia

1. Development of SOP on CarraPGP
2. Storage study on CarraPGP
3. Completed the study on determination of optimize CarraPGP dosage application (80 – 100 ppm) depend of plants.
4. Application of 0.1% of SWA decreased 50% of water irrigation for vegetables.
5. Combination effect of SWA-PGP was observed on vegetables. Further study on how to increase yield (application dosage, fertilization etc)

Mongolia

1. To test synergistic effect of PGP and oligochitosan, PGP and Tebutin (fungicide)

Philipines

1. Retrogradation effect on the stability and swelling capacity of PAA/Starch SWA with storage
2. Reformulation of the PAA/Starch SWA to control retrogradation and improve stability with storage

Gaps in basic aspects

Malaysia

1. CarraPGP solution easily deteriorated at various condition even with preservatives.
2. No ready for up scale due to this property

Mongolia

1. No or limited facility available
2. SWA is not tested in field study.

Philipines

1.Difficult to conduct experiments and have meetings due to COVID-19

2. Delays in delivery of purchases and out of stock goods

## Gaps in application aspects

Malaysia

1. Shortage in budget and labor for field experiments.
2. Little acceptability of the technology by farmers/end users.

Mongolia

1. No or limited facility available
2. SWA is not tested in field study.

Philippines

1. Limited irradiation facility (Philippines)
2. Need technology takers or industry partners

Implementation plan

Malaysia

1. To approach farmers and industry. Submit proposal for fund application for field test and market study.
3. To complete the storage study. The study will be focused on type of preservative and concentration and get advice from Philipines to overcome this problem.
4. Up scaling process will be carried out after get the optimize property of CarraPGP which able to sustain its quality for long storage period
5. To establish and produce procedure of PGP application. No extra cost and no extra work load are factors should be concerned in this study.

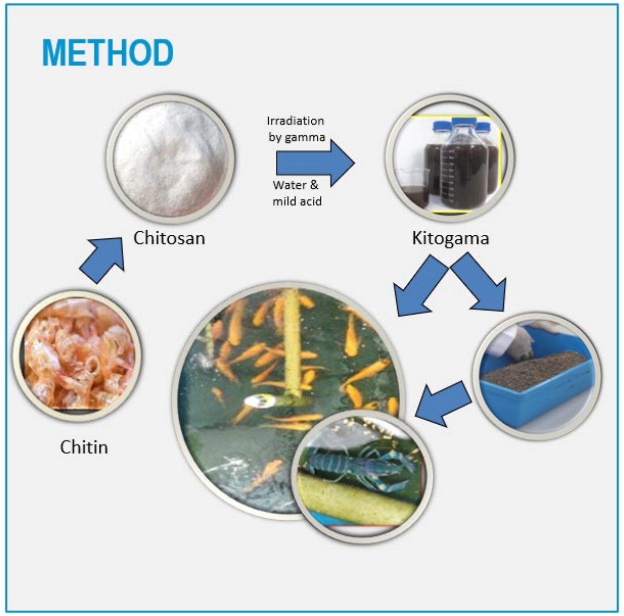
Mongolia

1. Develop to further modification of biofertilizer
2. Investigate impact of bacterial fertilizer on soil fertility

Philipines

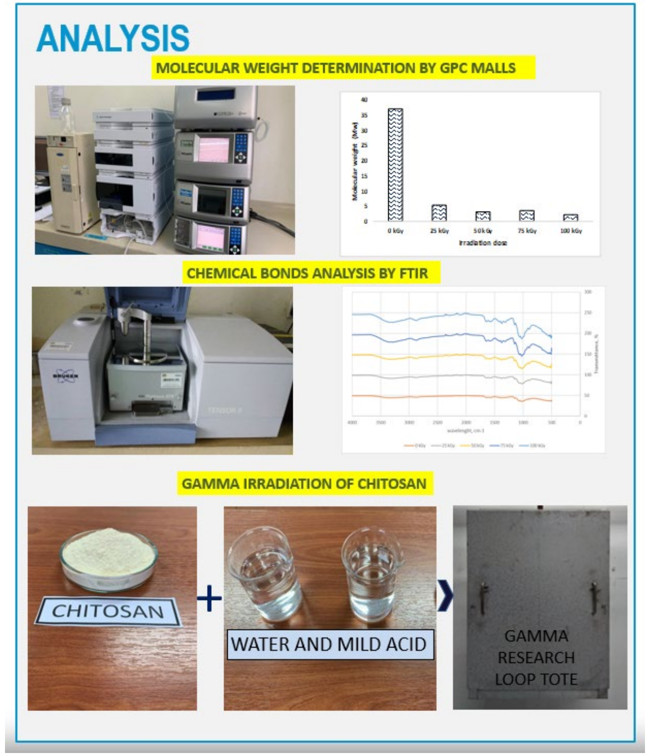
Submit proposal for the continuation of project and conduct new studies on CMC SWA incorporated with fertilizer and nematicide as delivery system.

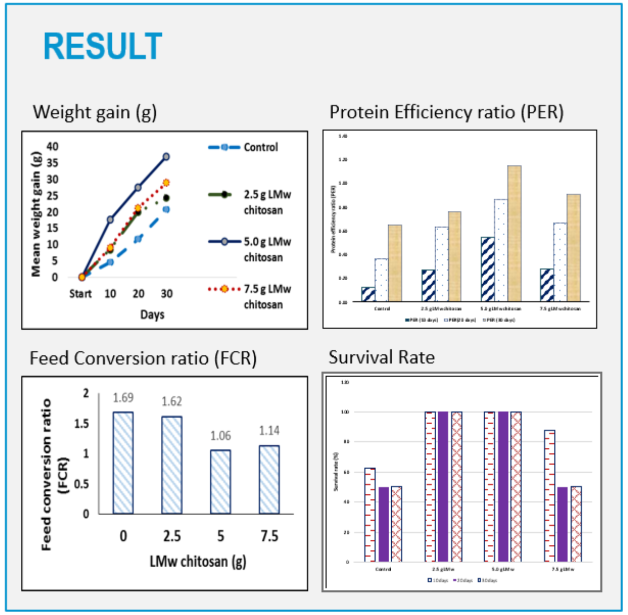
# DEGRADED CHITOSAN FOR ANIMAL FEED




1. Preparation of Kitogama (degraded chitosan for animal feed) is established.

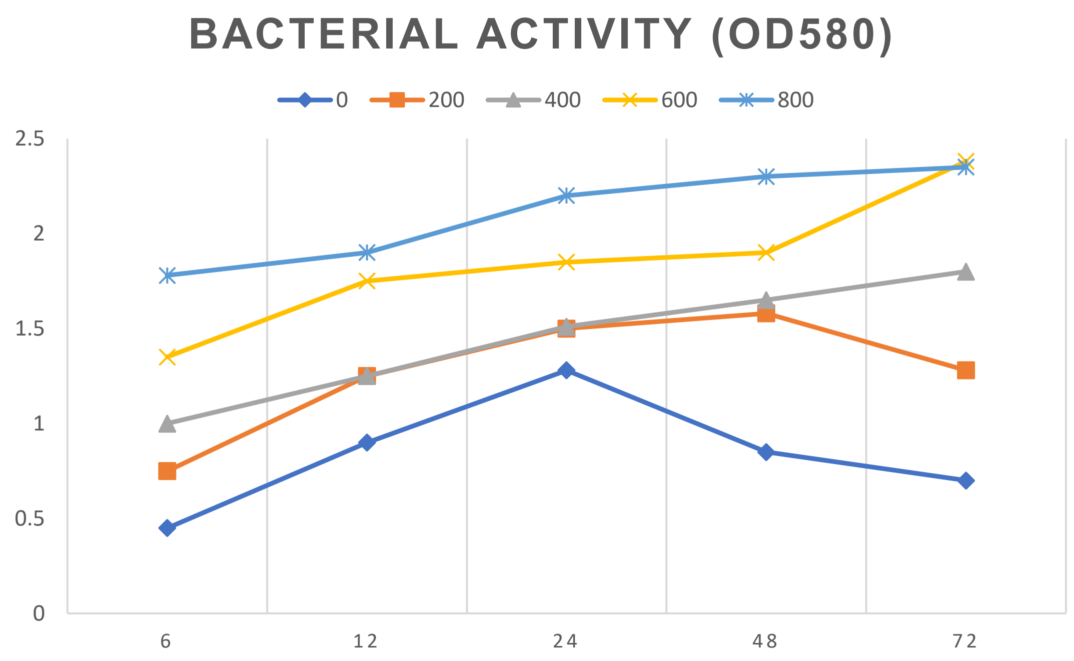

3. We identified method of application of Kitogama, by (i) pour into the water tank, (ii) add in the feed (fish feed and silage)



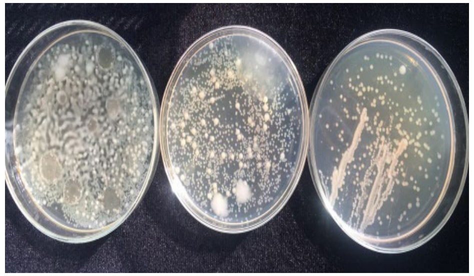




4. Study the effect of Kitogama on tilapia (2020), lobster (2021), chicken (2021) and cow (2018).

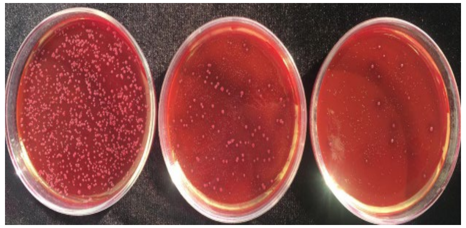

2. Quality assessment/control for every batch of raw materials and product to produce quality Kitogama.




** Determination used the spectrophotometer (at 580 nm)*



Azospirillum&azoarcus on NA



Bacteria on NA with tebutin of maximum dose

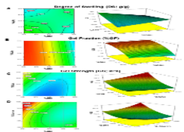
- Tebutin (fungicide) as seed fertilizer has no significant impact on bacterial activity.
- In average yield of 2021-2022, bacterial fertilizing plots were 6.9-9.0 centner/ha higher than control.

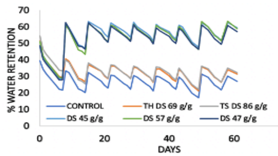
# R&D area: 7. PGP and SWA inclusive process development (2022)

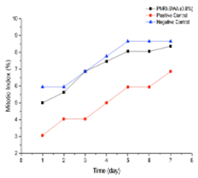
## Group member: Charito T. ARANILLA (Philippines)

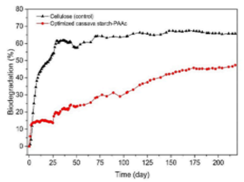
## Optimization

- **Formulation-** *20% AAc, 30% DN, 7.5% starch @ 20 kGy*
- **Biodegradation** - *46% in 218 days*
- **Safety evaluation** - *no genotoxic and chromosomal aberrations in mitotic activity*
- **Stability** - *9 months @ RT*
- **Retro-degradation**



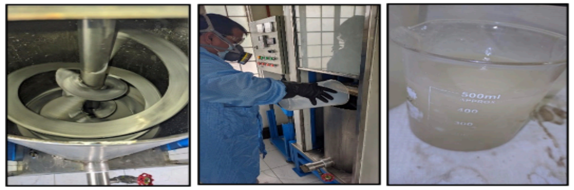


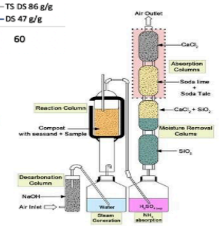



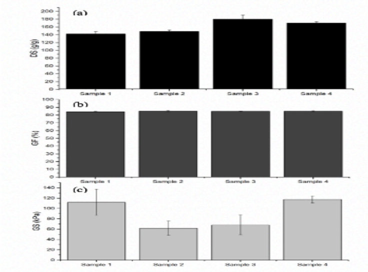




## Lab-Pilot Scale Production

- 1.5 L to 40 L SWA solution
- 5 kg dry SWA per production
- no apparent depreciation of gel properties from lab to upscaled production



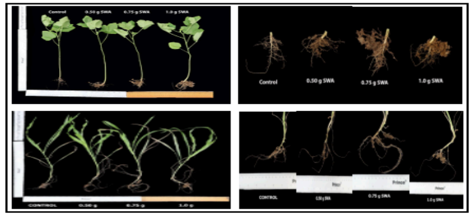



## Efficacy

**Major findings:**

- Pot: increase in root biomass and reduction watering interval, every 7 d to every 11 d
- Field: induced early flowering; reduced watering interval



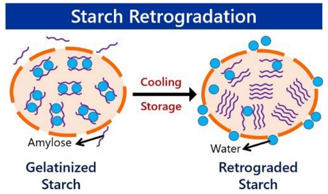
## Cost-benefit Analysis

- Competitive price with other biodegradable SWA
- Savings of 25-30% in cost of production.

| Market | Brand of SWA | Type of SWA | Selling price (PhP/kg) |
|---|---|---|---|
| | Terrasorb (USA) | Non-biodegradable (chemical) | 2,000.00 |
| | Newsorb (Japan) | Non-biodegradable (chemical) | 1,600.00 |
| | Alsta Hydrogel | Non-biodegradable (chemical) | 930.00 |
| | (India) | Non-biodegradable (chemical) | 620.00 |
| International | Stockosorb (India) | Non-biodegradable (chemical) | 200.00 |
| | Demi (China) | Non-biodegradable (chemical) | 160.00 |
| | Sungo (China) | Non-biodegradable (chemical | 160.00 |
| | TINT (Thailand) | Biodegradable (radiation) | 1,100.00 |
| Local | Zeba (Ph) | Biodegradable (Chemical) | 1000.00 |

Table 33. Cost-benefit analysis for lettuce and rice production at J.C. Dots per one cropping cycle and per hectare

| Items | Without SWA Application | With SWA Application (during intensely hot weather condition) | With SWA Application (during average weather condition) |
|---|---|---|---|
| Irrigation Frequency (per week) | 5 | 3 | 1 |
| Irrigation Frequency (per cycle, 42 days/cycle) | 32 | 19.2 (<40% vs w/o SWA) | 6.4 (<80% vs w/o SWA) |
| Irrigation Water | Free | Free | Free |
| Irrigation Water Cost | Free | Free | Free |
| SWA Application Rate (Kg SWA/ha)* | N/A | 8 kg/ha | 8 kg/ha |
| SWA Application Cost | N/A | Php 7,930 | Php 7,930 |
| Fuel Cost (Php 500/ irrigation) | Php16,000 | Php 9,600 | Php 3,200 |
| Watering Labor Cost (manpower) | Php 22,400 | Php 13,440 | Php 4,480 |
| Production Operating Cost (from land preparation to harvest) | Php 38,400 | Php 30,970 | Php 15,610 |
| Total Savings (Difference of production operating cost with SWA application–and production operating cost without SWA application) | N/A | Php 7,430 | Php 22,790 |




- Effect of retrogradation in SWA – significant decrease in the degree of swelling

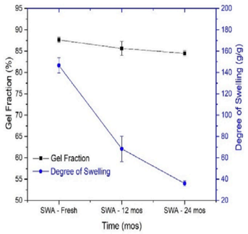
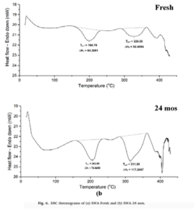
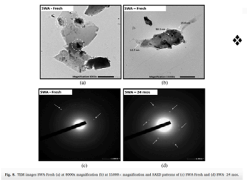



Fig. 2. Gel properties of SWA samples at different storage times.

- Significant increase in the enthalpy at the melting temperature of the starch suggest an increase in degree of crystallinity of the SWA



- SAED patterns of SWA-24 mos. depicted patterns of bright spots which corresponded to the formation of high order of starch crystallinity after 24 mos. storage time

| Modification/Additives | Initial Swelling (g/g) | Rate decrease in swelling/mo | Shelf-life |
|---|---|---|---|
| 1.5% bentonite | 117 | 19.0 $mo^{-1}$ | 2 mos |
| 2.5% bentonite | 136 | 20.0 $mo^{-1}$ | 3 mos |
| 2.5% CMC | 90 | 14.0 $mo^{-1}$ | 0 mo |
| Pre-irrad starch -100 kGy | 320 | 29.2 $mo^{-1}$ | 8 mos |
| Pre-irrad starch -75 kGy | 338 | 20.6 $mo^{-1}$ | 1 y |
| 2.5% $Na_2CO_3$ | 158 | 6.4 $mo^{-1}$ | 5.5 mos |
| 2.5% $CaCl_2$ | 227 | 3.0 $mo^{-1}$ | 3.2 y |
| Refrigeration 4 ºC | 170 | 1.4 $mo^{-1}$ | 3.5 y |
| Freezing | 170 | 0.22 $mo^{-1}$ | 23 y |
| Optimized SWA at RT | 170 | 6.4 $mo^{-1}$ | 9 mos |

# R&D area: Group G: 7 Sterilization and Sanitization Using Radiation (2022)

**Group member:** Dr.PHUA CHOO Kwai Hoe (Malaysia), Ms.Julieta A. Anarna (Philippines), Dr.Kunlayakorn Prongjunthuek (Thailand), Dr.Chinzorig RADNAABAZAR (Mongolia)

## Current status <Improvements from 2019>

All FNCA Biofertilizer members agreed that sterilization of carrier by using gamma irradiation improves the quality of biofertilizer and prolongs the shelf life of biofertilizer inoculant. However, the availability and accessibility of gamma irradiation for sterilization of biofertilizer carrier in some countries should be improved .

- **(Malaysia) :** The digestate was proposed as a new carrier for biofertilizer product. Digestate was sterilized at doses of 0 kGy to 50 kGy. Carrier sterilized at 50 kGy was free of microbes.
- **(Philippines) :** The Philippines is now using gamma ray sterilization at 20kGY in large scale production for sustainable production of Bio N microbial inoculant.
- **(Thailand) :** A new carrier (eucalyptus shell) sterilization by gamma ray at 25kGy is suitable for some microbial that use to produce PGPR biofertilizer. Until now Thailand also use non sterile carrier.
- **(Mongolia)** : Due to effects of X-ray irradiation bacterial counts were significantly reduced from $4.1*10^6$ to $2*10^5$ colony forming units after 5 kGy irradiation.

## <Remaining/New Challenges>

- New biofertilizer isolates for carrier biofertilizer development
- Gamma irradiation for sterilization of new carrier with out contamination and can use with all microbe

## R&D area: Group G: 7 Sterilization and Sanitization Using Radiation (2022)

**Gap in basic aspect**

- Cost of sterilization
- Only one gamma sterilization facility in Malaysia
- **No or limited facility available**
- **Lack of awareness on gamma irradiation technology**

**Gap in application aspect**

- ✓ **Price /cost of irradiation, Gamma irradiation is more expensive than conventional sterilization (steam sterilization)**
- ✓ Availability of facility
- ✓ distance of gamma irradiation facility. Far from the end user (Biofertilizer manufacturer)
- ✓ **Misunderstanding of gamma irradiation**, farmers are afraid to use something that had been irradiation by radioactive. Also, in organic farming and good agriculture practice (GAP) of Thailand can not use irradiation materials

**Implementation plan**

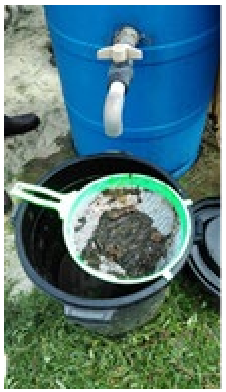
- Reduce the carrier size for sterilization (reduce the cost of sterilization) (Malaysia)
- Post-harvest treatment of basic vegetables (potato, carrot, garlic, onion) with irradiation to reduce losses during preservation (Mongolia)
- Promote and educate farmers and small businesses, provide nuclear technological information to the public as a safe (Mongolia)
- Use of lower doses of Gamma irradiation for sterilization (Philippines & Thailand)

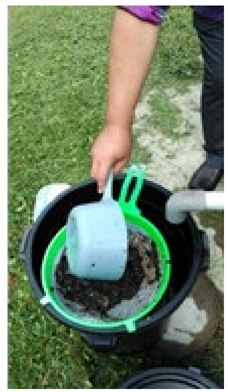
Group member: Dr. PHUA CHOO Kwai Hoe (Malaysia)

**Project Title:** Gamma irradiation for carrier sterilisation

**Objective:** To optimised gamma irradiation dose for carrier sterilization

**Summary:** Gamma sterilization of new carrier done. Dose 50 kGy free of microbe.

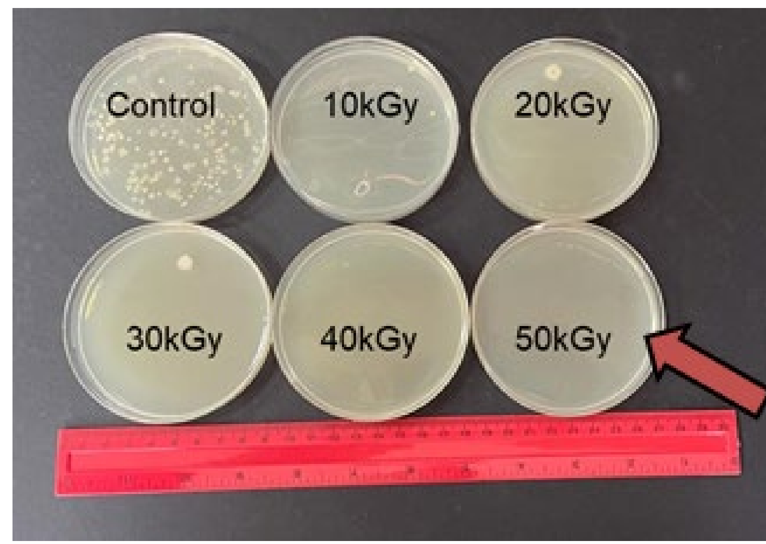




Digestate was produced by fermenting organic waste (i.e. food waste) anaerobically in a biodigester.


Gamma irradiation for carrier sterilisation


Carrier irradiated at various doses




Dose 50kGy free of microbe

Group member: Dr. Chinzorig Radnaabazar (Mongolia)

**Project Title:** Gamma irradiation for carrier sterilization

**Objective:** To improve hygiene of Mongolian traditional product curd.

**Summary:** Ionization irradiation has beneficial effects to prolong the storage period and reduce microbial contamination in .


After irradiation of 5kGy absorbed dose:

- Total bacterial count is reduced to $2*10^5$
- No changes of chemical composition (FT-IR)
- Moisture decreased 22%
- The color, taste and odor not changed
- Protein and lipid content decreased not significantly

Group member: Ms.Julieta A. ANARNA (Philippines)

## Comparison of using gamma irradiation (20kGy) technique with heat autoclave method for sterilization of carrier (FNCA Project)

| Particulars | Irradiated Carrier | Heat autoclave carrier |
|---|---|---|
| Efficiency | 1.7 tons per week (10,303 packets) | 1 ton per week (5,000 packets) |
| Texture of the Carrier | Dry and ready to use | Wet and laborious |
| Cost per packet | Php1.30(.03$) | Php1.03(.02$) |
| Lifespan | 10 months | 6 months |

Group member: Dr.Kunlayakorn Prongjunthuek (Thailand)

# Development and Dissemination of Radiation Sterilization Method of New Types of Carrier


## Eucalyptus shell

- Soaked in water 10 years
- Dried and grinded with fine grinding
- Sieved through 2 mm sieve
- Moisture < 20%
- pH 7.1-7.3

Sterilization of the carrier used in the production of PGPR-I influence of growth and survival of all three genera. It should be studied and developed methods of sterilization suitable for all three genus and other. Also, from the results gamma ray at 25kGy for carrier sterilization is suitable for *Azospirillum* sp. and *Beijerinckia* sp.

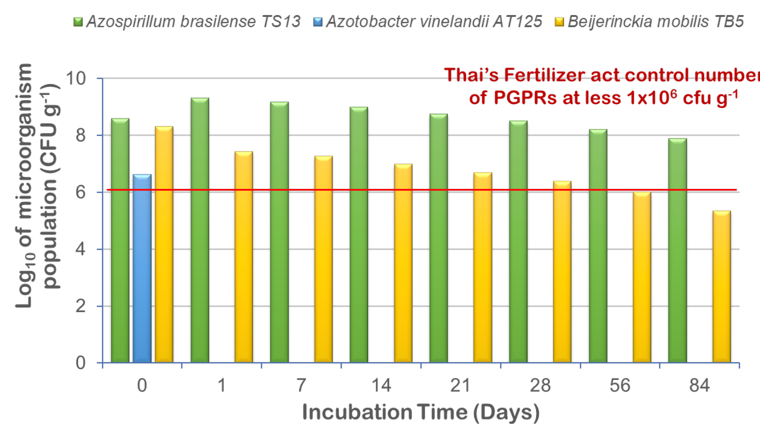



Figure 5: Survival of microorganism in a sterilization by γ-irradiation at 25 kGy carrier (T4)

R&D area: Group H: 8. Plastic Recycle (2022)

Group member: Dr. Tamada Masao (Japan)
Dr. Tita Puspitasari (Indonesia),
Dr. Suwanmala (Thailand)
Dr. TRAN Minh Quynh (Vietnam)
Dr. Charito Aranilla (The Philippines)

Current status

(Indonesia) The development of new products based on recycle plastics were elaborated. There are two kinds of products such as compatibilizers for wood plastics composite (WPC) as mechanical recycling and fuels as chemical recycling mechanisms. All assisted by radiation processing. Regarding development of WPC, the result shows that the formation of radicals between rPE and TTKS (OPEFB) during irradiation will bind the molecules more tightly to each other so that the interaction between the matrix and the filler is higher. Furthermore, the preliminary study in terms of thermal decomposition on the degradation radiation process to obtain fuel from rPP combined with zeolite supplementation on pyrolysis reveals that the irradiated PP with zeolite supplementation decrease the decomposition temperature due to the combination of cracking via radical and catalytic mechanisms (via proton addition, beta scission (β-scission, etc.)

(Vietnam) Radiation crosslinking have been applied to improve thermal stability and mechanical properties of bio-plastics include starch, PLLA composites for further applications. Prepare recycle plastic filters, which combined the special catalysts to degrade the wastes and biofilms formed after filtration.

# R&D area: Group H: 8. Plastic Recycle (2022) (Continue)

## Current status

(Thailand) Thailand is working on a project titled “Fabrication of recycled plastic composites with neutron shielding properties”. The project aims to use turn local plastic waste (caps of water bottle, mostly HDPE) into composites with neutron shielding properties. The concept of the project has been proven by both a number of published research work as well as a fact that there are similar products commercially available in the market, mostly China and USA. Preliminary experiments and results have also shown that composites made from recycled HDPE can offer neutron shielding properties. The project is currently in TRL 2 and are undergoing processes to finish TRL 3 at the end of 2022, at the earliest. The experts (from the IAEA) offer a recommendation for the project to use compounds (made from recycled plastic) produced by private companies to make sure that the raw material can offer more consistent properties. Hence, a collaboration with private companies has been initiated. CirPlas is an SME company in Thailand who is collecting plastic wastes and turning them into compounds to be used to create different products. CirPlas will be the official supplier of the raw material for Thailand’s project.

(Philippines) New project titled Post-radiation Reactive Extrusion Proof in connection with NUTEC project; Identified ENVIROTECH Philippines as private partner for the technology; Conducted proof of concept experiments at TARRI-QST

## Remaining/New Challenges

- Adaptation with the new business process in BRIN in terms of financial support, facilities access, administration support, and networking with external agencies especially the utilization of the R&D product and tech. (Indonesia)
- Optimization of radiation and laboratory processes
- Laboratory scale production of actual size products
- Validation of performance requirements according standards

## Gap in basic aspect

- Human resource replacement, facilities ageing, basic concept should be proven and, the product should meet with the technology and economic feasibility (Indonesia)
- Lacking of key facility for polymer processing (internal mixer, compression molding machine) and mechanical characterization (universal testing machine); Delays in delivery of purchases and out of stock goods (All)

## Gap in application aspect

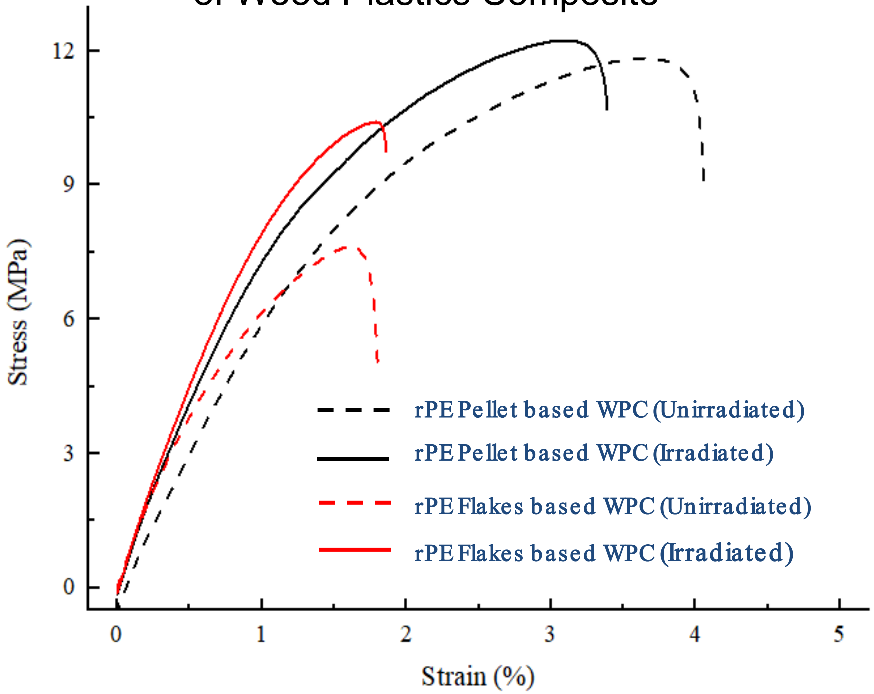
Economic feasibility
Properties of the product compare with the commercial products.
Need technology takers or industry partners for sustain collaboration

## Implementation plan

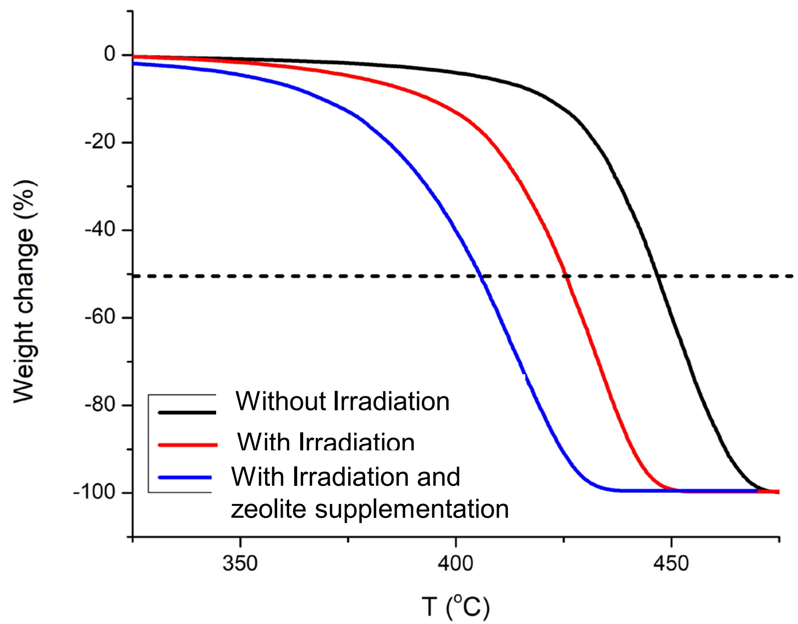
(All) The collaboration with private company and university to carry of the research and development on recycle plastic by radiation processing in order to have the starting material and facility for polymer processing and mechanical testing machine.
(The Philippines) Submitted proposal for project funding
R&D collaboration with ITDI-DOST and ENVIROTECH Philippines

# Indonesia

Effect of Irradiation Dose on Mechanical Properties of Wood Plastics Composite



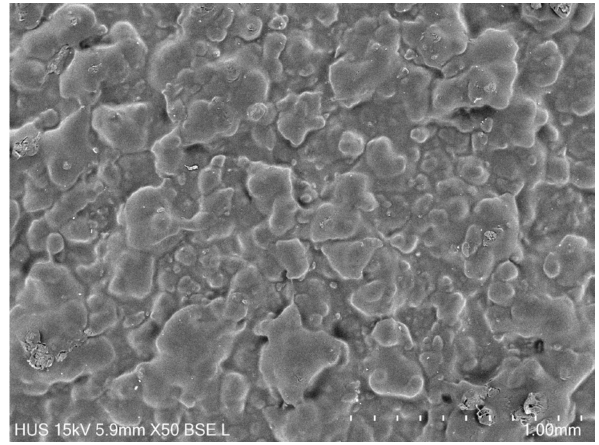

Effect of irradiation and zeolite supplementation on the decomposition temperature of the Degraded Polypropylena



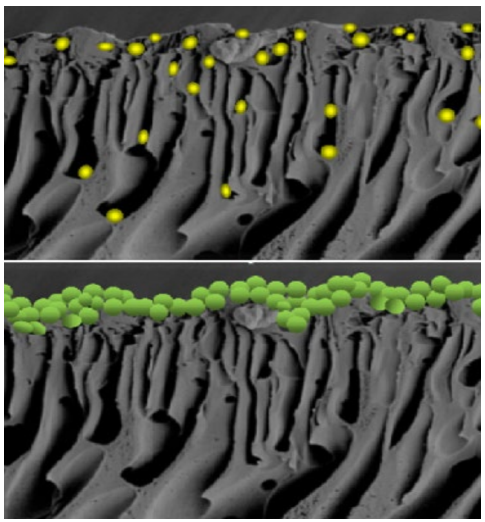

- The formation of radicals between rPE and TTKS (OPEFB) during irradiation will bind the molecules more tightly to each other so that the interaction between the matrix and the filler is higher.
- The interaction between the matrix and the high filler will increase the surface adhesion properties of the two materials leading to efficient stress transfer from the matrix and fiber so as to make the composite more compatible.

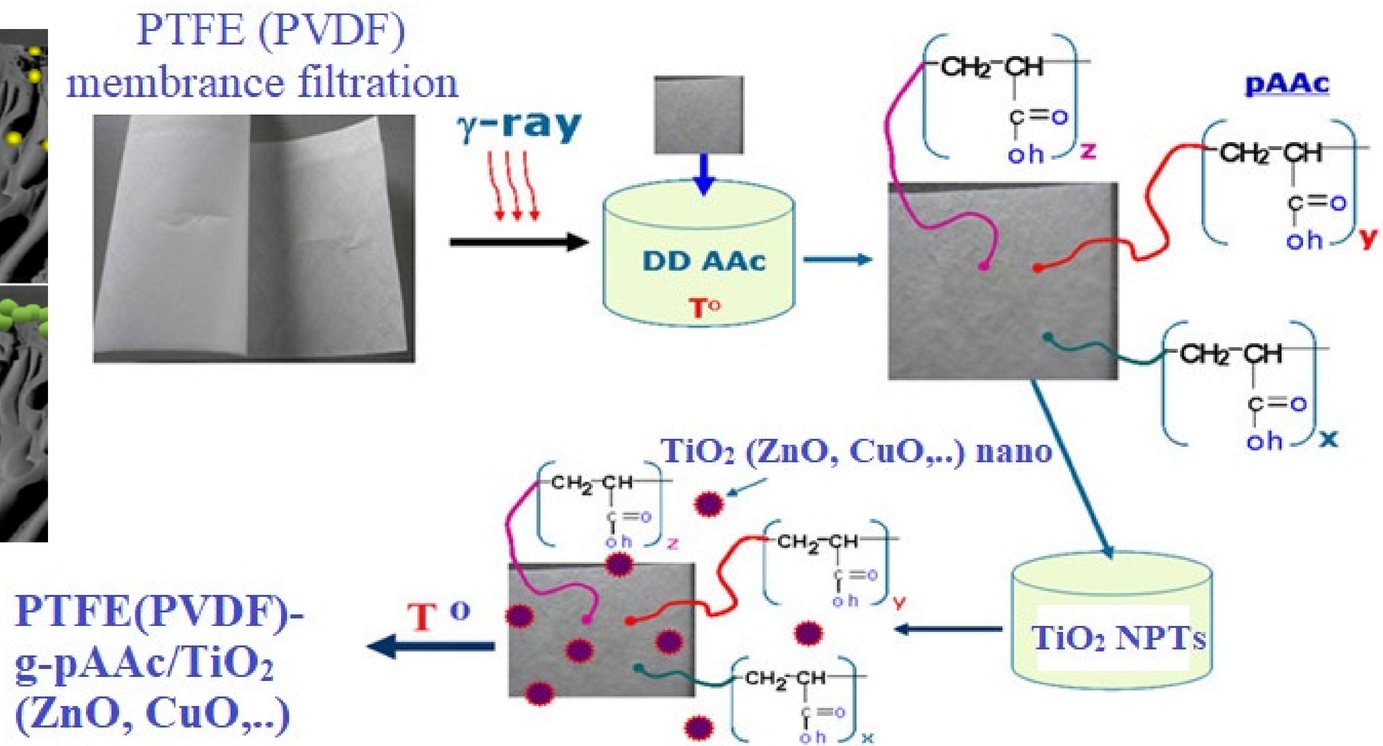
The thermogravimetric (TGA) thermogram reveals that the irradiated PP with zeolite supplementation increased the decrease in the decomposition temperature due to the combination of cracking via radical and catalytic mechanisms (via proton addition, beta scission (β-scission, etc.)

Vietnam


1. PBAT/starch films for industrial applicartions



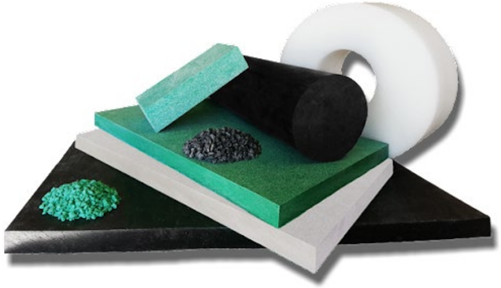



2. Prepare the membrane that can remove all pollutants by radiation modification, and try to apply in practice to remove the dyes from wastewater.

Thailand

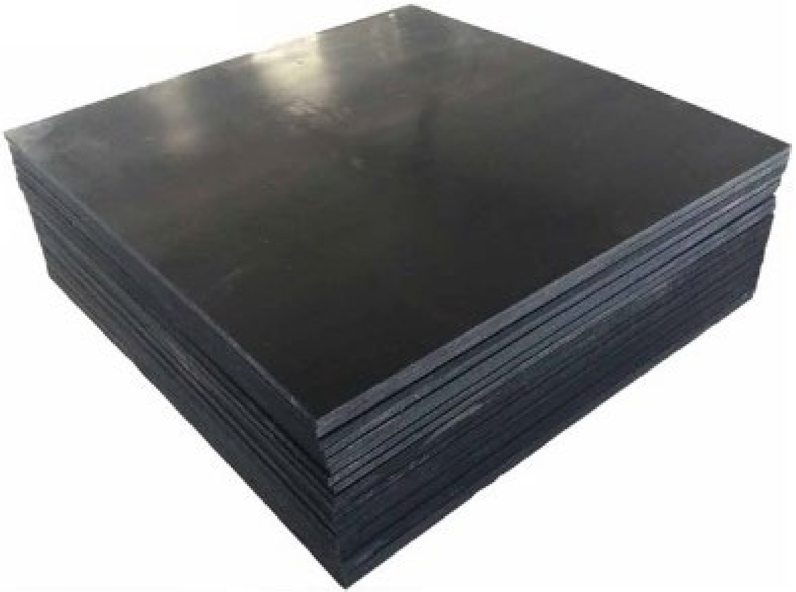
## Planning for 2023

**To reduce plastic waste by recycling them into light-weight materials with neutron shielding property**



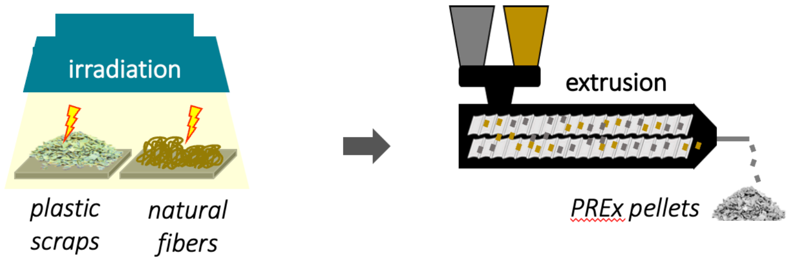
## Borated PE Sheets: Status in Thailand

- Currently, there is no manufacturer in Thailand
- Commercially available only by import (China, USA)
- PE loaded with Boron for neutron shielding purpose
- Import: High cost (especially for order with low volume)
- Price for 13 Sheets = 500,000 THB = 13,000 Euro



(Philippine)

## Post-radiation Reactive Extrusion (PREx)




**Radiation**
Formation of radicals, oxidative species, formation of crosslinks and grafting, control of melt index

**Extrusion**
Supports the reactive species in enhancing miscibility and/or inducing crosslinks