Session Summary of 2019 FNCA Radiation Processing and Polymer Modification Project Workshop

Session Summary

Session 2: Overview and Achievements of FNCA Projects

1) FNCA Achievements 2018-2019 (Mr Tomoaki Wada)

FNCA Ministerial Level Meeting agreed to encourage the member countries to bring the R&D products of such projects as mutation breeding, radiation processing and polymer modification to the end-users including the private sector, promote cooperation with international organizations, and further accelerate FNCA activities not only by accelerating the existing R&D themes but also adopting possible future R&D themes. Regarding Radiation Processing and Polymer Modification project, we have started discussing the new R&D themes such as animal feed supplement, environmental remediation, and hydrogel for medical application since last year. Interesting R&D reports have already being made form member countries.

2) Project Overview (Dr Masao Tamada)

Radiation processing and polymer modification project was launched newly from 2018 by merging electron accelerator and biofertilizer projects. The project explored gaps in basic and application aspects and corresponding implementation plans in the following seven R&D subjects which meet the needs in participating countries:

1. Degraded chitosan for animal feeds
2. Hydrogel for medical application
3. Environmental remediation
4. Synergistic effect of plant growth promoters (PGP), super water absorbents (SWA) and biofertilizer (BF)
5. PGP and SWA inclusive process development
6. Mutation breeding of BF microbe using gamma irradiation
7. Sterilization of BF carrier using gamma irradiation

Special topics toward technology transfer in participating countries are:

- Marketing authorization of oligochitosan as immuno-stimulant and growth in aquaculture was issued. (Degraded chitosan for animal feeds, Vietnam)
- More than 150 patients were treated by hydrogel wound dressing (Hydrogel for medical application, Bangladesh).
- Adsorbent for collecting uranium from seawater was prepared on a bench scale and evaluated by seawater waterway experiment. (Environmental remediation, China)

We will continue information sharing and discussion on seven subjects that will lead various applications in agricultural, environmental, and medical field to promote R&Ds and their technology transfer to end-users in participating countries.

Session 3: Progress Report on Biofertilizer

1) **Dr Md Kamruzzaman Pramanik, Bangladesh Atomic Energy Commission, Bangladesh**

1. Synergistic/ combined effect of chitosan (as PGP) and *Azopirillum spp.* (as biofertilizer) on rice plants

Combined effect of chitosan as PGP and *Azospirillum* species as biofertilizer on rice plant was studied in a semi-field level experiment.. Six treatments were applied with triplicates viz, T_1: 100% chemical fertilizer, T_2: 40% chemical fertilizer, T_3: 40% chemical fertilizer +100ppm chitosan, **T_4:** 40% chemical fertilizer +100ppm chitosan+ biofertilizer, T_5: 40% chemical fertilizer + biofertilizer and T_6: Control (native nutrient). Rice variety, BRRI-129 was selected as test rice variety and several parameters including tiller height and number, panicle length and grain yield were assessed to determine if any synergistic or combined effect of chitosan and biofertilizer is present. Result showed that both the highest tiller height (93.98 cm) and tiller number (17.89/hill) were found in T_1 and no synergy was found with respect to these parameters. The highest panicle length (24.46 cm), grain no./panicle (194.12) and straw weight were also found in the same (T1) treatment and were almost unaffected with either treatment. Grain size was increased upto 3.07% in the T_4-treatment as measured by 1000-grain weight but not at significant level. Grain yield of rice (t/ha) was increased up to 4.41% in T_4–treatment in comparison to control but not as synergistically. Overall results indicate that integrated use of chitosan (100ppm) plus biofertilizer along with (40%) chemical fertilizer has some effect with respect to grain size and yield.

2. Incorporation of antimicrobial activity in PVA hydrogel upon addition of chitosan

Hydrogel made of polyvinyl alcohol (PVA) is a material that has potential and different biomedical usages including wound healing and burn dressing. PVA along with other natural polymer (e.g., k-carrageenan) is usually processed by gamma radiation and produces flexible, transparent, mechanically suitable, economical and biocompatible hydrogel. In Bangladesh, hydrogel produced/ processed by ionizing radiation has been used for several years which is made of only PVA or PVA plus k-carrageenan where radiation processing and sterilization is performed simultaneously. As chitosan is a natural polymer and its antimicrobial activities can be obtained or enhanced by applying gamma radiation, an effort was made to incorporate chitosan in PVA to attribute the gel with antimicrobial properties upon irradiation by gamma ray from Co-60 source. In this experiment 2.0% and 1.0% chitosan dissolved in 2.0% and 1.0% acetic acid was mixed with same amount of 20% PVA dissolved in distilled water to produce mixture of 1.0% and 0.5% chitosan in 10% PVA, respectively. After casting, this material was irradiated with a radiation dose of 25.0 kGy from Cobalt-60 gamma source. Then, gel disk was made with sterile borer and tested for antimicrobial activity against both Gram positive (*Staphylococcus aureus*) and Gram negative (*E. coli*) bacteria by disk diffusion method on Mueller Hinton Agar medium. Result showed that gel containing chitosan showed antimicrobial activity as per concentration dependent manner as measured by zone of inhibition and Gram positive bacteria was found more sensitive than Gram negative bacteria. Beside antimicrobial properties, some of the physical parameters (e.g., swelling ratio, porosity etc.) were also improved in chitosan- incorporated PVA hydrogel. However, further test and trails are required to optimize and confirm the suitability and biocompatibility issue before its final applications.

2) Dr Nana Mulyana, National Nuclear Energy Agency, Indonesia

Climate change and mismanagement of natural resources was affect to land degradation. This condition can disrupt the sustainability of agriculture and the environment, so that a comprehensive effort is needed to restore the land strategic function. Land bioremediation is an effort to improve the plant's rhizosphere ecosystem. The use of local organic matter and inoculant selected functional microorganisms is very necessary in land remediation with extreme environmental stress. Selected microorganism strains can be obtained through exploration or increased ability of selected strains from culture collections. Co-60 gamma irradiation has the potential to be used in increasing the ability of selected strains, especially selected fungi strains. The use of Co-60 gamma rays has also been proven to produce carriers with the high strility and quality guarantee. Land bioremediation with inoculant functional microorganisms and local organic material is expected to be an alternative solution to develop the rhizosphere ecosystem and restore the land strategic functions

3) Biocontrol and Growth Promotion Effects of Bacillus-based Biofertilizer with Oligochitosan (Dr Shin Okazaki, Tokyo University of Agriculture and Technology, Japan)

We screened plant growth-promoting strains from field soils at the Tokyo University of Agriculture and Technology (Tokyo, Japan) and isolated a strain, TUAT1, which was identified as *Bacillus pumilus*. TUAT1 promotes the growth of several plants, including that of rice and *Brassica* species. An inoculant for rice was developed using TUAT1 that has been commercialized as “Kikuichi” in Japan.

Meanwhile, we found that TUAT1 could inhibit the growth of some plant fungal pathogens when cultivated together in a agar plate. To understand the biocontrol activity and mechanisms of *Bacillus pumilus* TUAT1 on different plant fungal pathogens, we evaluated biocontrol spectrum and identify the biocontrol agents of TUAT1. We found that TUAT1 could inhibit the growth of several fungal pathogens. Among them, we found that TUAT1 could inhibit strongly the growth of *Calonectria ilicicola* which causes soybean root rot disease, one of the severest soybean disease in the world. On the pot experiment, we found that the TUAT1 could alleviate the disease occurrence of soybean root rot disease and promote the growth of soybean. Furthermore, additional treatments of soybean plants with oligochitosan together with TUAT1 could enhance the the disease control as well as the seed production. We further analyze the biocontrol agent produced by TUAT1 and for understanding the biocontrol mechanisms and further application.

4) Current Status and Future Research of Malaysia’s Biofertiliser (Dr Phua Choo Kwai Hoe, Malaysian Nuclear Agency, Malaysia)

Malaysia’s biofertiliser market is currently focusing on liquid multifunctional biofertiliser products. Numerous farmers, especially paddy growers, have been in search of biofertiliser products. Various paddy plots had been tested with biofertiliser and had shown favourable yields. The Malaysian Nuclear Agency had commercialised a multifunctional biofertiliser (Bioliquifert). Two multifunctional biofertilisers, namely, AP1 and M99, are currently in the process of commercialisation. Improvements in the multifunctional activities of biofertiliser microbes had been conducted through gamma irradiation. Two gamma-irradiated biofertiliser microbes, namely, *Acinetobacter baumanii* and *Acinetobacter calcoceticus*, had been obtained.

The mutation effects on nitrogen and phosphate solubilisation gene (*nif* and *pqq* genes) had been studied. The synergistic effect of radiation-processed chitosan with biofertiliser had been performed on maize and vegetable crops. No synergistic effect of radiation-processed chitosan with biofertiliser on vegetable crops was generated in the greenhouse experiment. The combined radiation-processed chitosan, biofertiliser with chemical or organic fertiliser on maize at plot experiments resulted in favourable yield in comparison with farmer practices. Gamma sterilisation on carrier showed that 20 kGy can kill bacteria, fungus and actinomycetes. Future biofertiliser research will focus on multifunctional biofertiliser, mutagenesis biofertiliser microbes, seed treatment biofertiliser through gamma irradiation breeding tomato seed, developing a cost-saving radiation-processed chitosan mixture biofertiliser and gamma sterilisation using a new biofertiliser carrier.

5) Study on The Effects of Oligochitosan and Biofertilizer on Greenhouse Plants (Ms Sunjidmaa Otgonbayar, Institute of Plant and Agricultural Sciences, Mongolia)

Evaluation of the synergy effect between bio-fertilizer and oligochitosan was conducted to determine the growth and yield of the tomato and pepper. Study was conducted in a split-plot design with 2 replications was used and each replication consisted of nine plants. Study for synergistic effect of biofertilizer and irradiated oligochitosan, some positive effect such as plant growth promoter in tomato and pepper growth stage in the green house experiment. The plots on Oligochitosan and biofertilizers application was high efficiency and more than green mass 30-35%, vegetative growth, flowering and fruit development stages was in before 5-7 days, rate to control.

6) Performance of *Bio N* microbial inoculant and Carageenan on the Yield of Corn (Zea maize) (Ms Julieta A. Anarna, National Institute of Molecular Biology and Biotechnology, the Philippines)

The heavy applications of chemical fertilizers result to disturbance of soil properties, environment and human health It is in this regard that bio-fertilizer research was undertaken come up with more cost-efficient and alternatives to imported chemical fertilizers for enhancing agricultural crops with less dependence on chemical fertilizer. The use of microbes as biofertilizer is an important part for sustainable production of agricultural crops. Biofertilizer is a cheaper alternative to the continuous rising cost of chemical fertilizer and can help boost crop production at a much lower cost and thereby increase farmers' income.

This year three experiments were conducted using corn as the test crops to determine the effect of single and combined inoculation of biofertilizers commercially available at BIOTECH-UPLB namely Bio N (Azospirillum)and Mykovam (Mychorhiza).Plant growth promoter carrageenan formulated from radiation-processed carrageenan—an extract from seaweed processed into powder .from PNRI was also evaluated in combination with Bio N. All experiments were set up under field condition at BIOTECH demonstration farm and in one of the province of the Philippinnes (Tanauan, Batangas laid out in a Randomized Complete Block Design (RCBD) with 4 replications, Different treatments were employed in each studies. For Study 1 Effect of chemical fertilizer and Bio N inoculation conducted from September to November, 2018 the treatments used were T1 - Full Chemical Fertilizer and T2 - Full Chemical Fertilizer + Bio N. For the study combined inoculation of Bio N and Mykovam the following treatments were used, T1 – Control T2 – Full Chemical fertilizers T3 – Full Chemical fertilizer + Bio N T4 - 1/2 Chemical fertilizer + Bio N T5- Bio N + Mykovam and T6 – ½ Chemical fertilizer + Bio N + MykovamFor synergy effect study the following treatments were employed in the study T1-Control, T2- Chemical fertilizer, T3- ½ Chemical fertilizer, T4- Bio N, T5- Bio N + Chemical fertilizer, T6- Bio N + ½ Chemical fertilizer, T7- Carrageenan, T8- Carrageenan, T9- Carrageenan+ Chemical fertilizer and T10- Carrageenan + Bio N Yield data was gathered and computed from the sample plots. Data obtained from study 1 shows positive effect with 29 .22 % increase over the uninoculated plots. The results showed on study 2 that mean weight of corn from the plants treated with ½ chemical fertilizer combined with Bio N and Mykovam were comparatively similar to the fully fertilized plot .The study revealed that the highest yield was obtained from plots treated with both Bio N and carrageenan with 15.96 tons and 15.87 tons per hectare respectively compared with chemical fertilizer with only 14.16 tons per hectare. A 1.80 tons was realized due to inoculation and application of Bio N and carrageenan. The application of biofertilizers whether singly or with co- inoculation improve productivity of the test crop. The National Institute of Molecular Biology and Biotechnology is continuously promoting the use of biofertilizers and extending it to the Filipino farmers. Sterilization of Bio N BF carriers using 20kGy gamma irradiation is being employed.

7) Development of Liquid Rhizobium Bio-fertilizer and Phosphate Solubilizing Bio-fertilizer Powder by Spray Drier (Dr Kunlayakorn Prongjunthuek, Department of Agriculture, Thailand)

Powdered rhizobium bio-fertilizer faced with the problem of contamination in carrier. Therefore, studied in a liquid form which is convenient for use because it can be used to mix seeds directly without seed coat or sticky substances. Studying liquid formulations for rhizobium bio-fertilizers of mung bean containing various polymers such as CMC, soluble starch and MgO using YM as a standard formula and product retention period. The results showed that rhizobium can grow well in YM + MgO 1 g/L, with the amount of survival after 180 days of storage at room temperature at 2.18×10^9 colonies/ml.

Phosphate solubilizing bio-fertilizers using cow manure fermented as carrier, which is uneven and contaminated. Therefore, develop the production model in powder form by spray drying machine by selecting highly effective bacteria, the optimum conditions for powder form production and storage of products was studied. The results performed that *Pseudomonas fluorescents* SM-P025B had the survival after spraying drying at 110 and 120^{o}C more than 80% and the product gave the highest of tomato germination at 92.31%. For storage period of 6 months at 4^{o}C and room temperature gave the amount of survived was 2.18×10^8 and 1.16×10^8 colonies/gram bio-fertilizer, respectively, which exceeded by the Fertilizer Act.

8) Preparation of Microbial Fertilizer in Beads for Vegetable (Dr Tran Minh Quynh, Vietnam Atomic Energy Institute, Vietnam)

In this experiment, a granular microbial fertilizer containing nitrogen fixation bacteria (*Azotobacter* *chroococcum* VACC 86) and IAA producing bacteria (*Bacillus megaterium* VACC 118) has been prepared. Briefly, the bacterial strains were selected from the Culture collection of Soils and Fertilizers Research Institute, activated and cultured in corresponding fermentation media. Then, the stationary phase cultures of *A. chroococcum* and *B. megaterium* at densities of about 7.5×10^{7} and 2.5×10^{10} CFU/g, respectively, were homogeneously suspended in the carrier mixture of 2% sodium alginate and 33% radiation modified cassava starch. Resulting solution was dropped in 2.5% $CaCl_2$ solution for precipitation in bead. The bead containing bacteria were stabilized by crosslinking between sodium alginate and calcium ion for further 30 min, then dried to a critical moisture below 10%. Density of surviving bacteria in the beads was determined according to TCVN 6166:202 and TCVN 8736:2011 periodically at days 7, 30, 90 and 180. The results revealed that the cell density in the resulting microbial fertilizer still higher than 10^{8} CFU/g after 6 month storage.

Resulting beads were applied to vegetable at the rate of 20 kg per ha and the results revealed that the fertilizer could promote the growth of cabbage, tomato and radish cultivated in both screen house and field. Application of the resulting fertilizer with NPK much increased the rate of folded plant, fresh weight of head and yield of the cabbages grown on alluvial soil. These results also observed in the plant treated with reduced inorganic fertilizer (80% NPK as local standard). Thus, the microbial fertilizer can be partly replaced for chemical fertilizers, as potential solution for sustainable agriculture in order to reduce environmental pollution and adapt to climate change.

Session 4: Progress Report on Polymer Modification

1) Mr Md Saifur Rahaman, Bangladesh Atomic Energy Commission, Bangladesh

1. Application of oligo-chitosan as plant growth promoter on Capsicum (*Solanaceae Genus)* and Strawberry (*Fragariaananassa)*

Nuclear and Radiation Chemistry Division, Institute of Nuclear Science and Technology, Atomic Energy Research Establishment, Savar, Dhaka, Bangladesh.

Semi-Field and pot experiments were conducted at the yard of Atomic Energy Research Establishment, Savar, Dhaka, Bangladesh during the period December 2018 to May 2019to investigate the effect of o-chitosan application on the growth and economic yield of capsicum and strawberry respectively. The foliar application of oligo-chitosan was done every ten days interval up to harvesting. Effects of oligo-chitosan on both the plants' growth and productivity were investigated in terms of total number of fruits, total weight of fruits, harvest time and % yield. For Capsicum, the experiment comprised of four (4) levels of oligo-chitosan concentrations viz., 0 (control), 50,75 and 100 ppm. The results showed that the foliar application of oligo-chitosan at 75 and 100 ppm concentrations displayed significant effects. The productivity was increased up to 89 and 146 % over control for75 and 100 ppm respectively. In the pot experiment for the strawberry plants the used oligo-chitosan oligo-chitosan concentrations were 0 (control), 25, 50, 75 and 100 ppm. Among all the concentrations 25 and 75 ppm showed significant results. The results showed that the productivity of the strawberry plants increased upto 54 and 48 % for 25 and 75 ppm respectively. For both the plants under experiment, oligo-chitosan shortened the harvest time in contrast to control. These results suggest that foliar application of oligo-chitosan at 75 or 100 ppm for Capsicum and 25 or 75 ppm for Strawberry can be taken as the optimum concentrations for maximizing plant growth and yield of these plants.

2. Preparation of hydrogels dressing from PVA and Chitosan with improved properties by gamma radiation.

Our group is working on the preparation and characterization of hydrogels from various hydrophilic polymers by the application of gamma radiation. A hydrogel from 10% PVA and 1% k-carrageenan for biomedical application by gamma radiation (25 kGy) and have been applying this excellent dressing material for burn wounds at Uttara Adhunik Medical College Hospital, Uttara, Dhaka, since March 2011 for clinical test. We are trying to supply the hydrogels to other medical colleges as an effort of expanding the arena and acceptabilit of the this hydrogel. The feasibility study of the hydrogels at 20, 30 and 40 ºC showed that, the use of hydrogel is feasible over the experimented temperatures. The routine sterility check of the hydrogels are done per batch from Microbiology and Industrial Irradiation Division, Institute of Food and Radiaiton Biology, BAEC. As an effor to enhance and improve the quality of the hydrogel dressing we have prepared chitosan incorporated hydorgels by gamma radiation at 25 kGy radiation dose having 10% PVA with (A) 1, (B) 0.5% (1% acetic acid),0.5% (0.5% acetic acid) and (D) 0% chitosan contents. Gel fraction and crosslinking density of the hydrogel containing 1% chitosan is the lowest (90 %) and the % swelling ratio (~500%) of this hydrogel is highest. Between the hydrogel samples B and C, B renders lower % gel (~95%) and thus higher % swelling ratio (~400%). Antimicrobial activity of the prepared samples were done at Microbiology and Industrial Irradiation Division, Institute of Food and Radiaiton Biology, BAEC and positive result was found for the all the samples. Further investigation is required to optimize a chitosan containing hydrogel for medical use.

2) Develop of Metal Ions Adsorbent and Super Water Adsorbent with Radiation Induced Graft Polymerization and Crosslinking (Dr Ma Hongjuan, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, China)

A series of fibrous adsorbent was prepared by pre-irradiation grafting of monomers including acrylic acid, acrylonitrile, glycidyl methacrylate et al. onto the polymeric fiber using ^{60}Co γ-rays and electron beam irradiation. The original and modified fibers were characterized by a series of characterization methods to demonstrate the attachment of functional groups onto the fibers. The adsorption capacity of the functional fiber was investigated in aqueous solutions containing various metal ions such as Cr(VI), Cd(VI), U((VI)) et al.. The breaking strength confirmed that the fibrous adsorbent could maintain good mechanical properties and long service life.

Amylum-based super water adsorbent (SWA) was prepared with co-irradiation induced grafting and crosslinking of acrylates. Water absorption ratio can research 350 g/g in deionized water and 50 g/g in 0.9wt% NaCl aqueous solution, which meet a criterion of Chinese agricultural industry standards. This SWA is using in west China for desertification control. Survival rate of the typical plant in desert was significantly enhanced with certain content of SWA.

3) Application of Irradiated Chitosan for Plant Elicitor and Animal Feed Additive (Dr Darmawan Darwis, National Nuclear Energy Agency, Indonesia)

National Nuclear Energy Agency (BATAN) Indonesia has successfully prepared irradiated chitosan (oligochitosan) from shrimp shells using demineralization, deproteination, and deacetylation process to produce chitosan and it was followed by irradiation using gamma rays. It is well known that oligochitosan is used as PGP (plant growth promoter), plant elicitor, animal food additive, pharmaceutical and cosmetic product, as well as biomedical materials for application in medicine. In this report, oligochitosan was used for PGP, and plant elicitor to suppress the rust leave disease on the Chrysanthemum plant and as an animal feed additive for ruminant (cow and sheep) and hen. In the Chrysanthemum plant, oligochitosan was foliar sprayed with a concentration of 100 ppm once a week for 3 months. As a positive control, the Chrysanthemum plant was sprayed with Hyponex and Extragreen liquid fertilizers. While for animal feed additive, oligochitosan was added to the concentrated animal feed with a concentration of 300 up to 500 ppm and without oligochitosan added to the concentrated animal feed as a control. The animal was given the formula every day up to 40 days. The results showed that the Chrysanthemum (PN variety) treated with oligochitosan have shorter harvesting time (112.33 days) compared to the Chrysanthemum treated with Hyponex (121.33 days) and Evergreen (123 days). It was observed that oligochitosan suppressed rust leave disease *i.e.*, having the disease of only 21.53% compared to control (36.13%). Application of oligochitosan as animal feed additive showed increasing body gain in Pasundan's cow with the increasing of concentration of oligochitosan. It was showed that the increasing of body gain after 40 days application of oligochitosan with concentration of 0, 300, 400 and 500 ppm are 5.2, 9.8. 10 and 13.8 kg, respectively. The same tendency was also observed for increasing body gain in sheep and hen. Additionally, the oligochitosan increased the weight of hen's egg. It can be concluded that oligochitosan is effective as PGP and plant elicitor to suppress rust leave disease in Chrysanthemum plant and increase body gain of Pasundan's cow, sheep, and hen.

4) Preparation and characterization of hydrogels by electron beam cross-linking (Prof Takehisa Hanawa, Tokyo University of Science, Japan)

Oral formulations are frequently used in pharmacotherapy because of their convenience, but drugs with a bitter or unpleasant taste may cause lower adherence. Reduction of bioavailability by the hepatic first pass effect is also of concern for oral preparations. On the other hand, transdermal absorption-type pharmaceuticals are odorless, can avoid the hepatic first pass effect, and are applicable for patients with undeveloped swallowing function such as infants or elderly patients with dysphagia. Transdermal formulations, in particular, have the advantage of being able to be immediately discontinued by peeling off when side effects occur. However, the skin permeability of drugs depends on the condition of the skin, and because of individual differences, techniques and drug carriers to improve skin permeability have been examined and developed.

Recently, gel formulations have attracted attention as one of the transdermal-absorbable formulations. Gels are semi-solid formulations capable of incorporating hydrophilic or hydrophobic solvents in the interstices of a three-dimensional network, and are classified into hydrogels containing water and organogels containing organic solvents based on the nature of the liquid phase within the gel.

Hydrogels can retain a large amount of water in a three-dimensional network, and are used as absorbent bodies for paper diapers, soft contact lenses, water retention materials for plants, etc.. Hydrogels are also more suitable for medical materials by having structures closer to biological tissues than other synthetic biocompatible materials, and are expected to be further exploited. Examples of practical applications as medical hydrogels include hydrogel-type wound dressings that promote healing by wetting wounds and disastrous exudate absorbers that absorb excess exudate.

Common preparation methods for hydrogels include chemical and physical cross-linking methods. The chemical cross-linking method involves adding chemical cross-linkers, such as glutaraldehyde and 1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N',N'-dicyclohexylcarbodiimide (DDC) to form covalent cross-linking of polymeric chains. However, EDC and DDC are highly toxic to living organisms, causing severe skin allergy, and skin irritation, such as cytotoxicity, due to residual cross-linkers, and rash and redness caused by organic solvents are problematic; therefore, a safer preparation method is required. On the other hand, physical cross-linking methods without the use of cross-linkers include the freeze-thaw method, by which the local concentration of polymer chains increases locally to form cross-linked structures by noncovalent bonds occurring between polymer chains because ice and macromolecules are phase separated by cooling the aqueous polymer solution below the freezing point, the γ-ray irradiation method and the electron beam irradiation method, by which cross-linking, degradation and polymerization occur due to radicals generated by the polymer undergoing γ-ray or electron beam irradiation. The electron beam cross-linking method may be able to prevent toxicity problems due to residual cross-linkers because the hydrogels are formed by irradiating water-soluble polymer solutions, such as hydroxypropyl cellulose (HPC) and methyl cellulose (MC), with electron beams, and no cross-linker is used. However, although hydrogels formed by irradiating HPC and MC solution with electron beams are currently utilized as wound dressings, those formed from hydroxypropyl methylcellulose (HPMC), a cellulosic derivative, have not been reported. When a hydrogel is used as a drug carrier, there is a method of introducing a drug into the hydrogel by immersing the drug solution in the hydrogel for a long period.

Therefore, in this study, we focused on tramadol hydrochloride (TRA) as a water-soluble drug. TRA is used as an analgesic for several cancers and as a postoperative analgesic. It acts on μ-opioid receptors by inhibiting the reuptake of serotonin and noradrenaline, and is freely soluble in water [19]. In this study, we first prepared hydrogels from water-soluble polymeric HPMC by electron beam cross-linking and assessed their physical properties. Then, the potential applications of HPMC hydrogels prepared by electron beam cross-linking in transdermal absorption-type formulations were investigated using the water-soluble drug TRA as a model drug.

Irradiation of aqueous HPMC, a water-soluble polymer, with electron beams formed hydrogels at both concentrations (10 or 20%) and all electron doses (10, 30 or 50 kGy). Furthermore, the hydrogels formed by electron beams exhibited different physical properties depending on the electron beam irradiation dose. For the water-soluble pharmaceutical TRA, its release from the hydrogel and skin penetration differed depending on the irradiation dose. Therefore, the released amount of the water-soluble drug TRA was able to be controlled by changing the irradiation dose.

5) Dr Pavel Krivtsov, JSC "The Park of Nuclear Technologies", Kazakhstan

Kazakhstan considers relevant to use super water absorbers (SWA) because of farming in areas with a shortage of fresh water. Therefore, the Park of Nuclear Technologies has been conducting studies since 2014 and what's more, it has established SWA pilot production from potassium polyacrylate. The PNT has produced a pilot batch of SWA in the amount of 150 kg and transferred it to Krasnovodopadskaya experimental station and forestry Semey Ormany for field research. The following results were obtained: chickpea yield increased by 180% after using SWA 25kg/hectare compared to the control sample which increased by 196% using SWA 50 kg/hectare. The survival rate of two annual pine seedlings was raised twice.

The PNT is conducting research to raise carboxymethylcellulose in SWA; it obtained a patent for a method of producing SWA using electron accelerators; registered BetaSorb trademark; purchased and established equipment for SWA production with a capacity of 200 tons/year. Production line is going to be launched in 2019.

Investigations are also underway on using SWA in the manufacture of substrate for mushrooms' production. SWA can reduce loss of moisture by substrate that leads to an increase in productivity. When using SWA from CMC, SWA will also be a breeding ground for fungal spores in addition to keeping.

6) Radiation Modification of Polymers Current R&D&C Activities in Nuclear Malaysia (Ms Maznah Binti Mahmud, Malaysian Nuclear Agency, Malaysia)

Nuclear Malaysia (NM) is the leading government institute in R&D&C of products produced by radiation processing technology in Malaysia. The application of radiation technique becomes one of the established tools in bio and synthetic polymers modification in order to enhance properties as well as the functionalities of the polymers besides chemical and physical techniques. Currently NM is working on synergistic studies involving oligochitosan/SWA and oligocarageenan/SWA on Chinese kale (pot test scale) and oligochitosan-liquid fertilizer (M99) on papaya and corn (field test scale). Results showed that combination treatments give positive impact to germination rate, growth rate and quality of yield. NM also has collaborated with local companies through R&D&C cooperation towards commercialization of oligochitosan as PGP. NM has taken a step forward to introduce oligochitosan as food supplement for animal feed. As for now, we are focusing for fish feed and currently still in progress to produce oligochitosan with 5000 Da molecular weight to meet the requirement for this purpose. NM has taken initiative to collaborate with Fishery Research Institute in order to make the fish feed project succeed. Furthermore, NM continuing the study on development of hydrogels towards the application as drug carrier and 3D cell culture matrix. The hydrogels consisting of biosynthetic polymers indicate the hybrid system of radiation-induced-crosslink of chitosan-PEG-PVP and PVP-carrageenan hydrogels are biocompatible and suitable for cell proliferation and growth.

7) Biological Remediation of Physical Degraded Soil with Plantation and Bacterial Mixed Culture (Dr Chinzorig Radnaabazar, National University of Mongolia, Mongolia)

Problems in Mongolian mining include deficient remediation measures and a lack of consideration for environmental issues. In 2011, used mining sites were 20401.3 hectare and remediated land was 4630.3 hectare in phase one, 4587.6 hectare in phase two which is biologically remediated. Overall statistics indicate that only 22.4% of mining are is properly remediated and there is dozens of abandoned mine land.

The objective of this study was to investigate synergy effect of several species of plants (*Medicago varia,* *Stipa sibirica, Astragalus adsurgens, Allium mongolicum* et al.,) and mixed bacterial culture in physically degraded soil at gold mining area. Depending our result we indicate bacterial composting is effective and plant vegetation, survivability was higher than bare soil.

8) Radiation-Modified Polymeric Materials for Various Applications (Ms Charito Aranilla, Philippine Nuclear Research Institute, the Philippines)

The Carrageenan PGP was recently launched in various locations in the Philippines by two DOST-PNRI licensee companies (MTPSI and HTLTCI) under the brand names Vitalgro and Aqua Oro. Both licensees have plans for expansion and putting up their own facilities. In the meantime, they use the PNRI e-beam facility for their initial requirements for product sampling and inventory for dealers. The DOST-PNRI Licensing Agreements for MTPSI and HLTCI were signed in 2018 and each had remitted a non-refundable technology transfer fee of 1.5M pesos or 28,000 US dollars. Super water absorbents (SWA) based on cassava starch and acrylic acid (AAc) were synthesized based on FNCA guidelines. Synthesis optimization using a statistical design software generated optimum parameters in terms of component ratio, degrees of neutralization (DN) of AAc and irradiation doses and these parameters were verified experimentally. Based on water retention efficiency in sandy loam-clay-rich soil, SWA with formulation of 20% AAc (30% DN), 7.5% starch and radiation dose of 20 kGy was the most efficient. Radiation-crosslinked hydrogels based on carboxymethyl cellulose and k-carrageenan/polyethylene oxide were prototyped into hemostatic granules and dressing to control bleeding in traumatic wounds. These hemostatic agents can be used to improve emergency response and increase survivability in trauma victims in military battlefields, disasters, household accidents and in medical operations. Both hemostatic agents have no cytotoxicity and acute systemic toxicity, non-irritant and are weak dermal sensitizer.

9) The Applications of Radiation Processing in Thailand (Dr Phiriyatorn Suwanmala, Thailand Institute of Nuclear Technology, Thailand)

Over the past 10 years, Thailand has invested in facilities that have enabled it to apply radiation processing technology for peaceful purposes. Radiation processing has been utilized in various fields including: industry, environment and agriculture. The facilities include Gamma and Electron beam radiation.

In agriculture, the radiation-induced sterile insect technique (SIT) has been utilized for the control of oriental fruit fly populations in provincial orchards. A reduction of 80-90% fruit fly population was achieved in the targeted areas. TINT conducts research to investigate the effects of gamma and electron beam irradiation on the microbiological quality of herbs, as well as to characterize the functional components, antioxidant activities and other related functional assay. Gamma radiation and electron beam treatments can reduce or eliminate microbial contamination. The use of irradiation for food quality and safety has been applied to help Thailand export six types of fruit to the United States of America (USA). Gamma radiation has also been used successfully to induce useful mutation in crops and ornamental plants. Radiation-induced degradation was used to reduce the molecular weight of prepared chitosan, yielding oligochitosan. The treatment of chili plants by oligichitosan clearly displayed positive effects on chili's growth and productivity. For the environment, radiation-induced grafting was used to prepare dye adsorbent and metal adsorbent for environmental application.

In industry, cosmetic products are being developed through exploitation of the ability of irradiation treatment to increase the yield of active components during the extraction process. Furthermore, polymeric materials, especially natural polymers such as starches, cellulose, chitin and chitosan, are natural polymers with high potential for various applications due to their unique properties, especially biodegradability and biocompatibility. The super water absorbents (SWA), derived from cassava starch and sugarcane bagasse by radiation processing, is used in agriculture. SWA acts as a local reservoir, releasing water vapor into soil and plants as needed and also maintains moisture balance. It is applied to relieve water deficiency in the arid areas of Thailand. Electron beam irradiation is used to enhance the color of gemstones for improving their appearance and value addition.

10) Synthesis of Radiation Crosslinked Gelatin/Carboxymethyl Chitosan Hydrogel Scaffold for Tissue Culture (Dr Duy Ngoc Nguyen, Vietnam Atomic Energy Institute, Vietnam)

The hydrogel scaffolds from biocompatible natural polymers have been investigated and developed for application in tissue engineering. In order to create a suitable hydrogel scaffold, the selection of polymer compounds and hydrogel production methods has always attracted many studies. Hydrogels from gelatin/CM-chitosan mixtures with the different weight ratios of 10/0, 9/1, 8/2, and 7/3 were prepared by γ-ray irradiation-crosslinking. After irradiating, the hydrogels were determined the gel fraction and equilibrium water swelling by the weighing; and scanning electron microscope (SEM) images of scaffolds were taken after freeze-drying to determine the porous structures. The results showed that the dose of 30 – 35 kGy is necessary to prepare the hydrogels with gel fraction of 72 - 84%; equilibrium water swelling of 4.9 – 12 g/g after 10 hours immersing; the porous size of 100 – 350 μm. Amongst all the studied samples, the hydrogel gelation/CM-chitosan with the ratio of 9/1 (13.5 g/1.5 g/100 ml water) attained the highest gel content (~84%), the water absorption degree (5.8 g/g) and porous size of 120 – 250 μm, met the requirements for use as a mesenchymal stem cell culture scaffold.

Session 6 & 7: Discussion/Presentation on Achievements, Obstacles and Planning

Participants divided into seven groups discussed achievements, gaps in basic and application aspects, and implementation plans for the following expected needs in the participating countries:

A) Degraded Chitosan for Animal Feeds
B) Hydrogel for Medical Application
C) Environmental Remediation
D) Synergistic Effect of Plant Growth Promoters (PGP), Super Water Absorbents (SWA) and Biofertilizer (BF)
E) PGP and SWA, Inclusive of Process Development
F) Mutation Breeding of BF Microbe Using Gamma Irradiation
G) Sterilization of BF Carrier Using Gamma Irradiation

Conclusions were as follows:

A) Degraded Chitosan for Animal Feeds

Current status

- Vietnam: established preparation method of oligochitosan for animal feed, field test finished, licensed oligochitosan as immunostimulant for fish and shrimp, to be continued by combination selenium nanoparticles and oligochitosan to increase immuno system of shrimp.
- Thailand & Malaysia : decided to work on preparation of oligochitosan for animal feed
- Indonesia: Established the preparation protocol for oligochitosan for animal feed, to be continued with combination of oligochitosan with animal feeds and will conduct experiment and find the optimum condition for the application.

Remaining/New Challenges

- Competitiveness with the commercial product in term of enhancing the productivity
- Fund constraint
- Registration – difficult to register as animal feed
- Vietnam – collaboration RI with third party to build up the factory and to get the license
- Public acceptance – conduct seminar/media/exhibitions in order to increase the public acceptances.

Gap in basic aspect

- The mechanism of oligochitosan in growth performance and immunostimulant on animals not clearly understood.

Gap in application aspect

- Competing with commercial product
- Comparison between oligochitosan and available commercial products not conducted yet

Implementation plan

- Thailand/Malaysia: will start the experiment
- Indonesia: will conduct the experiment using oligochitosan and animal feed and find the optimum condition for the application.
- Vietnam: collaboration RI with third party to build up the factory and to get the license, conduct experiment on combination of oligochitosan and selenium nanoparticles for shrimp

B) Hydrogel for Medical Application

Current status

- Introduced the treatment of neuropathy using hydrogel loaded with API (Tromadol HCl) (Japan).
- Shelf life study was done. (Bangladesh)
- Incorporation of antimicrobial activities using Chitosan. (Bangladesh)

Gaps in basic aspects

- Diffusion behavior of the drug molecules into the hydrogel. How to regulate the release behavior for applying in DDS.
- How to load the desired function into the hydrogel like pH, thermal sensitivity.

- How to load the the drug efficiently into the hydrogels, especially the water insoluble APIs.
- Physical and chemical properties of the hydrogels.
- Pharmaco-kinetic and dynamic properties.
- Pharmacological effects study for getting the pathogenetic data.

Gaps in application aspects

- How to introduce ethical issue while applying the hydrogels.
- Awareness among the benefactors and also to the concerned authority about the blessings of the prepared hydrogel.
- Make the concerned authority aware about the fruitful application of the nuclear techniques for the preparation of the hydrogel to overcome their fear over the Hiroshima and Nagasaki Nuclear Bomb issue, Fukushima Accident.

Implementation plans

- Translational research including Human resources from administration, University personnel, Personnel from pharmaceutical companies to do concerted research works to make the path clear for finalization of the plan.
- Including the safety parameters as this is the first step while introducing the product.
- Collaboration to study the pharmacological effects.
- Collaboration with Doctors to produce awareness to the mass people.

C) Environmental Remediation

Current status

<Improvements from 2018>

- One industrial plant for textile waster treatment using EB irradiation is now running and the economic analysis is acceptable and competitive （China),
- Bioremediation study is improved from tree the plantation to mining site
  Phytoremediation with biofertilizer (Mongolia),
- Previous year’s study is extended (Vietnam),
- Experimenting with polluted soil of cadmium, hydrocarbon, biocontrol of phytopathogenic Fusarium species, gold mining sites and biologically fertilizing nutrient poor agricultural fields (Indonesia)

<Remaining/New Challenges>

- Only textile wastewater is under treatment by EB combined with biological method, other type of wastewater need further study.
- Adsorbents combine with traditional method should be studied, adsorption should be executed by practice of engineering in order to control the cost.

Gaps in basic aspects

- Remove experimental variations and keep consistency.

Gaps in application aspects

- China:Reduce the cost of metal ion adsorbents.

- Mongolia:Funding opportunity is limited.
  Competition with traditional methods.
- Vietnam: the distribution of textile factory is not centralized, so it is to difficult for collect wastewater dyeing.

Implementation plans

- China:Scale up adsorbents for metal ion removal and uranium extraction.
  Radiation combine with other methods to reduce the cost.
- Mongolia:Collaborate with other researchers.
- Vietnam: Combination electron beam and biological method to treatment wastewater textile dyeing in large scale for 2 years (2020-2021)
- In Mongolia, overall statistics indicate that only 22.4% of mining are properly remediated and there are dozens of abandoned mine land. Successful implementation of bioremediation experience and methods should be distributed to other researchers.
- More than 5 g uranium have been extracted from natural seawater in China. Adsorbents will be scaled up to 10 kg and 100 g uranium will be extracted in next year.
- First time in Vietnam textile's factory waste dye polluted water is completely degraded using electron beam method in lab scale.
- In Indonesia, increased amount of yields (20-30%) were harvested from biologically remediated agricultural field of paddy rice.

D) Synergistic Effect of Plant Growth Promoters (PGP), Super Water Absorbents (SWA) and Biofertilizer (BF)

Current status

- Bangladesh: Conducted synergy study of PGP and Azospirillum in semi-field level using rice as test crop. Results did not show synergistic effect rather showed some combined effect on yield.
- Indonesia: Conducted synergy experiment in semi-field level using white pepper plant; result showed combined effect rather than synergistic effect.
- Japan: On pot experiment, bacillus-based biofertilizer plus oligochitosan applied on soybean could alleviate the disease occurrence of soybean root disease and promote the growth of soybean. The study showed synergistic effect in controlling disease.
- Philippines: Conducted field experiment on the synergy of BioN and Carrageenan PGP using corn as test crop. Results did not show conclusive synergistic effect on yield.
- Mongolia: On pot experiment, synergistic effect of oligochitosan and biofertilizer on sweet pepper and tomato greenhouse trial. Results showed high synergistic effect on yield.
- All members agreed that combined effect rather than synergistic effect in the yield of crops was more evident in the studies conducted. However, in the case of Japan, synergy was observed in terms of disease control.

Gaps in basic aspects

- Since there was no clear synergistic effect on yield but promising results on disease control (as presented by Japan), confirmatory experiments are recommended.
- Confirm synergistic effect of PGP and biofertilizer/microbes either in terms of:
  (1) Yield
  (2) Disease Control

Gaps in application aspects

- Shortage in funding and manpower.
- Experimental Uniformity

Implementation plan

- New experiments/repeated experiment to be carried out with some modifications.
- Recommended modifications:
  (1) Improve the viability of microbes
    - Mr Nana suggested to improve viability of microbes by culturing in the presence of magnesium and inoculating in carrier incorporated with silicate.
    - Silica enrichment of peat soil: 1:4 sand-peat soil
  (2) Application/Treatment
    - To mix oligochitosan and biofertilizer in the soil after conducting survival test
  (3) New experiments/ repeated experiment to carried out with some modifications.
    - Pot experiment / greenhouse trial on different crops (Mongolia)
    - Field experiments / synergistic effect of biofertilizer and oligochitosan on wheat and some vegetables (Mongolia)

Planning R&D in next phase (2021 – 2023)

- Develop finest biofertilizer with carrier material and commercialize (Mongolia)

E) PGP and SWA, Inclusive of Process Development

Achievements of PGP

- Bangladesh, Vietnam:
  1. Field Researches (Strawberry, Capsicum, Tomato).
  2. Extended collaboration with the commercializing wing BINA. And also BARI
  3. Extended collaboration with Agricultural University.
- Indonesian – Pilot production, Field Researches (Pepper, Chrysanthemums)
- Malaysia – Established the production and towards commercialization, start working in development of new PGP (oligocarrageenan)
- Philippines – Commercial production; Two commercial products out in the market
- Thailand – Semi - commercial production
- Mongolia – Research work

Achievements of SWA

<Field Researches>

- Philippines – none
- Thailand – Rubber Tree, Baby corn
- Kazakhstan – Chickpea, Scots Pine, Wheat

<Production Status >

- Philippines – Small volume
- Thailand – Ready for industrial production
- Kazakhstan – Ready for industrial production

<u>GAP in Basic aspect</u>

<PGP>

- Basic mechanism of plant growth promoter and elicitor
- Identification of the structure of carrageenan (PGP) – initial experiment was conducted using LC/MS technique. Results show fragments similar to the disaccharide unit of k-carrageenan but changes in the structure of the disaccharide cannot be elucidated. Further analysis will be done.

<SWA>

- Optimum condition to increase the biodegradability

<u>GAP in application aspect</u>

<PGP>

- Registration of the product in some countries.
- Field trial applications are not yet done in some countries.
- Shortage of funding
- Shortage of Manpower

<SWA>

- High drying cost
- High production cost
- Reluctance by possible end-users to change conventional practices and accept technology

<u>Implementation plan</u>

<PGP>

Applied Research:

- Expansion of PGP application in other crops e.g. leafy vegetables, fruits, legumes, corn, etc.
- Combination of PGP and biofertilizer.
- Extension of the research studies in the field levels.
- Extension of collaboration for getting rid of manpower shortage.
- Pursue to get rid of funding issue.
- Emphasize on the Data production and reporting from field trails for publication.
- Emphasize on the Commercialization.

<SWA>

Basic researches:

- Incorporation of agrochemicals into the SWA for slow release

- New biodegradable starting materials to increase biodegradability of SWA

Applied Research:

- Determine appropriate machine for mass production with the objective of decreasing production cost
- Testing on the combination of SWA + biofertilizer, SWA + mineral fertilizer

Technology Transfer

- Enhanced commercialization efforts
- More efforts on the promotion of the technology to end-users to increase its acceptance

F) Mutation Breeding of BF Microbe Using Gamma Irradiation

Current Status

<Achievements>

- Looking for multifunctional microbes, but each country have working on different species of microbes and different function

<Remaining/New Challenges>

- Looking for multifunctional microbes, but each country have working on different species of microbes and different function (High lignolytic activity, cellulolytic activity and chitinolytic activity)
- After screening of fungi mutant will be evaluation by PCR RAPD method to make sure that gamma irradiation have impact to in use mutant.

Gaps in basic aspects

- Gamma irradiation produced random mutant. By using ion beam the possibilities targeted mutant will be increased.
- After gamma irradiation will be produced random mutant so we must be screening about bioactivity of random mutant.
- Screening and selection methods conventional vs advanced (robotic) technique

Gaps in application aspects

- Different requirement for each country (bacteria sp., function, policy, acts etc.)

Implementation plan

- To check the stability of the mutants and get more better new mutants (Indonesia)
- Field experiments with mutant fungi (Indonesia)
- Synergistic effects of multifunctional microbes with oligochitosan (Malaysia)
- Pot experiment / greenhouse trial on different crops with SWA and PGP (Malaysia)
- Breeding of Trichoderma sp. and other bacteria with gamma irradiation (Vietnam and Thailand)
- Pot experiments and greenhouse testing of the mutants (Vietnam)
- Breeding some bacteria with gamma irradiation with Pot experiments/ greenhouse testing of the mutants (China)

Achievements in 2019

- 4 mutants of fungi: Phareochaete chysosporium, Trichoderma harsianum, Trichoderma viridie, Trichoderma reesei (Indonesia)
- Carrier sterilization, Commercialization of biofertilizer, Synergy effects of biofertilizer with oligochitosan and Mutagenesis of biofertilizer microorganisms and mutation breeding by using gamma irradiation (Malaysia)

Implementation plan for Next Phase (2021-2023)

- Develop fungi mutant inoculant with granular as bio control and plant growth promoting/ Commercialization (Indonesia)
- Technology transfer/ Commercialization (Malaysia)
- Develop multifunctional BF as biocontrol agent to control plant diseases (Malaysia)
- New PGP / oligocaragenan (Malaysia)
- Field tests and evaluation (China, Vietnam)
- Technology transfer/ Commercialization (Indonesia, Vietnam)
- Field tests and evaluation (China)

G) Sterilization of BF Carrier Using Gamma Irradiation

Current Status

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

Gaps in basic aspects

Lack of awareness of radiation technology in general and gamma facility in particular

Gaps in application aspects

- 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)

(Indonesia) : Effect of Gamma irradiation on chemical properties of carriers of microial inoculant

Findings:

Gamma irradiation for sterilization of inoculant carriers is much better than autoclave sterilization. Minor change in chemical properties of inoculant carriers while sterilization using autoclave changed chemical properties of carriers significantly and toxic to microbes, hence influence the survival of microbial in biofertilizer.

(Vietnam) : Improvement of Biofertilizer Carrier.

Findings:

Radiation modified starchs can be used as main component of the polymer carriers replacing for conventional carriers (peat, rice straw, bentonite, clay…). Water absorption of cassava starch improved by gamma irradiation at dose of about 5 kGy, and the resulting modified starch can be directly used as high quality carrier without sterilization.

(Philippines) : Application of Gamma Ray Sterilization of Bio N Carrier at 20kGY.

Findings:

The Philippines is now using gamma ray sterilization at 20kGY in large scale production for sustainable production of Bio N microbial inoculant. .

Implementation plan for Next Phase

- Indonesia, Vietnam and Philippines

  Encourage private company to engage and collaborate on R&D activities in order to produce the improved BF carriers. Establishment of new irradiation facilities both by the private and government sectors for the sustainable agriculture production.
- (Indonesia)

  Convince the Government (Batan) to prioritize and give more attention for sterilization using gamma irradiation.

  Develop a optimum dose database for different types of carrier material for biofertilizer.

  (Vietnam)

  Technology transferring to private irradiation company and improvement of e beam application for sterilization
- Philippines

  Search for better carrier that will require less dosage of Gamma Ray Irradiation Sterilization