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NM OLIGOCHITOSAN

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ABBREVIATION AND SYMBOLS LIST

ABBREVIATION	DEFINITION
NM	Nuclear Malaysia
LMC	Low Molecular Weight Chitosan
DDA	Degree of deacetylation
Mw	Molecular weight
QA	Quality Assurance
QC	Quality Control
FNCA	Forum for Nuclear Cooperation in Asia
NSRA	Nuclear Safety Research Association
SEC-MALLS	Size Exclusion Chomatography - Multi-Angle Laser Light
FTIR	Fourier-transform infrared spectroscopy
Da	Dalton
ppm	Part Per Million
γ	Gamma
PGP	Plant growth promoter
PE	Plant elicitor

1. NM OLIGOCHITOSAN

1.1 OBJECTIVE

This book is designed to elaborate on the production, application and related issues regarding NM-Oligochitosan research and development activities. Beside that, this document on NM-Oligochitosan may be used as guidance for researchers to improve the existing process and application of NM-Oligochitosan. Most importantly, this NM-Oligochitosan will act as an eye-opener to business players to realise the potential applications of NM-Oligochitosan and the importance to initiate collaboration with Nuclear Malaysia for the technology transfer and commercialization of this innovation.

1.2 INTRODUCTION

The development of plant growth promoter (PGP) from natural polymer by using radiation or nuclear technology was initiated in 2008 during the participation of Malaysia in Forum for Nuclear Cooperation in Asia (FNCA) organized by Nuclear Safety Research Association (NSRA), Japan. FNCA which consists of 11 member countries (Malaysia, Australia, Indonesia, Japan, Bangladesh, Kazakhstan, Mongolia, China, Thailand, Vietnam & Philipines) has decided to develop agricultural input from utilization of radiation processing technology (electron beam and gamma rays) in modification of natural polymeric sources. Natural polymers such as chitosan, alginate, carrageenan, cellulose and starch were subjected to radiation for modification. Malaysia has chosen to develop PGP from chitosan by using gamma ray in order to modify its chemicals and bioactivity properties. Besides, chitosan can also be obtained easily in local and international markets at low price due to its abundant sources. Chitosan is obtained from partial and full deacetylation of chitin using alkaline under certain reaction conditions. Chitin is a byproduct of seafood processing industry which is extracted from exoskeleton of crustaceans.

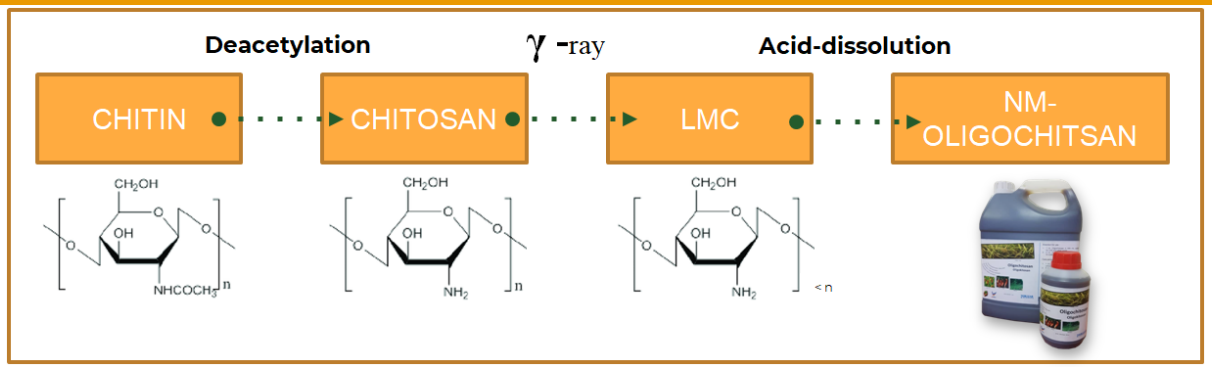

Figure 1.0 Production of oligochitosan from chitin

Gamma ray induces chain scission reaction in chitosan to produce low molecular weight chitosan or oligochitosan. **Figure 1.0** indicates the brief flow production of oligochitosan from chitin. The low molecular weight chitosan exhibits better performance as growth promoter and plant elicitor compared to unmodified chitosan which are usually consists of long chain chitosan with thousands Da (Dalton) of its average molecular weight (Mw). The gamma ray is an alternative green technology to modify chitosan compared to conventional techniques which involve various chemicals in the process that contributes to hazardous waste.

NM-oligochitosan consists of short chain chitosan prepared through the radiation-induced chain scission reactions. High dose of gamma ray is used to degrade long chain chitosan to shorter chain chitosan with average Mw ranging from 20kDa – 10kDa. Studies done on rice and various crops indicated that oligochitosan with Mw ranging from 10kDa – 20kDa increases the production yield. In addition, oligochitosan found to be effective as elicitor which increasing plant immunity against certain fungul and bacterial infections. In the next section, we will share the general methodology in producing NM-oligochitosan.

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2. METHODOLOGY

This section describes the general process of NM-oligochitosan production in Nuclear Malaysia. The production of NM-oligochitosan was established since 2010 using NM-

oligochitosan pilot plant facility located in RAYMINTEX Park, Dengkil, Selangor. It is equipped with a unit of two tonnes mixing tank with two-tier blade-type propeller, a unit of two tonnes collection tank and a suction pump. This pilot plant has the capacity in producing of up to two tonnes of NM-oligochitosan at a time.

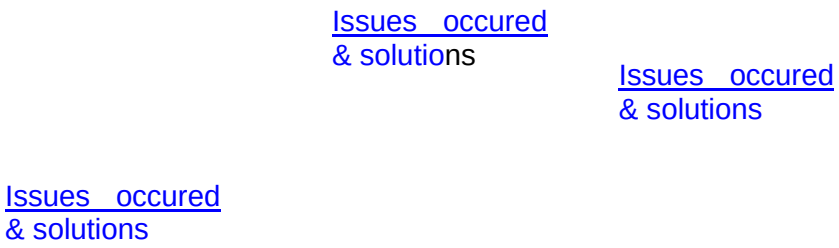

Figure 2.0 NM-Oligochitosan production process

Simplified process flow of NM-Oligochitosan production is shown in Figure 2. The production process of NM-oligochitosan begins with the irradiation of chitosan powder using gamma ray. The chitosan powder received from the supplier, exposed to the high dose gamma ray in the range of 50 – 100kGy depending on the initial Mw of chitosan. The irradiation process is carried out at ambient temperature and pressure without any chemical at SINAGAMA, MINT Tech-Park, Dengkil, Selangor.The irradiated chitosan powder characterized for Mw using size exclusion chromatography-multi angles laser light scattering (SEC MALLS) prior to mixing process. In order to retain its PGP function, the oligochitosan must exhibit a minimum of 85% deacetylation degree (DDA) value with the Mw of 10kDa – 20kDa.

The irradiated chitosan powder then dissolved in acidic solution and left overnight with gradual stirring in order to achieve homogeneous oligochitosan solution. A preservative agent is added on the following day. The protocols for production oligochitosan have been established. Detail of production process can be found from

SOP documents that can be obtained from Director of Radiation Processing Technology Division (BTS). The NM-oligochitosan has shelf life up to 2 years if it is kept dry and stored away from direct sunlight. This product also has pH value around pH 5which is suitable to soils and plants. Based on the field test experiences done with chili and rice, NM-oligochitosan offers less acidic condition to the seeds and soils which is favourable to the growth of seeds as well as to the environment.

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2.1 CHARACTERIZATION

Characterization of chitosan and NM Oligochitosan involves the measurement of Mw and DDA. The Mw of irradiated chitosan powder was determined by the SEC MALLS (size exclusion chromatography equipped with multi angle laser light scattering). This instrument is selected for Mw determination because it gives accurate and absolute Mw results. Determination of molecular weight is important as it determines the suitability of NM oligochitosan for certain application. For example, oligochitosan with a molecular weight of 10 kDa – 20 kDa and < 10kDa are suitable for PGP and PE, respectively as reported by Malaysia and Vietnam at FNCA Meeting 2015. Meanwhile oligochitosan with Mw ranging from 30 kDa-50 kDa is suitable for other purposes as reported by Massimo & Raffaella 2018. Method for molecular weight measurement with SEC MALLS can be found at Link.

Besides Mw, DDA also plays important role in determining the quality of chitosan. The higher DDA indicates better quality and functionality of chitosan. DDA can be determined by using various methods as mentioned by Biskup et al. 2012 ;Dandan et al. 2017 and Yonggin et al. 2011. Whereas NM chooses potentiometric titration and also FTIR for DDA determination.

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2.2 PRODUCT QUALITY MANAGEMENT

The purpose of product quality management is to maintain the chemical and physical properties of the NM Oligochitosan. To achieve this objective, the quality control procedure is implemented starting from analysis of incoming raw materials until the end stage of the NM Oligochtosan production. The NM oligochitosan which is produced in accordance with SOP NM Oligochtosan has two years shelf life. The issues, effects and solutions in relation to NM Oligochitosan production is summerized in Table 1.

3. APPLICATIONS

The treatment of various plants with oligochitosan has shown promising results in increasing agricultural yield through various means (root promotion, disease resistance and etc.). Pilot scale studies had been conducted using on various plants such as rice, chilli, vegetables and others for specific purposes through special techniques. Figure 3 show some successful stories and challenges of NM-Oligochitosan throughout the years of research and development.

Purpose

Enhancing seeds' germination, increasing seedling survivality, increasing yield & reducing disease infection.

Application on chilli farming

Dilute NM-OC to 100 ppm using water. Soak seeds for 2 hours and drip prior to planting. Spray twice a week (FNCA guidelines & Monirul et al. 2018).

Purpose

Enhancing seeds, germination rate, increasing tillering stage, reduce disease infection.

Application on paddy farming

Dilute NM-OC to 100 ppm using water. Soak seeds for 24 hours and 24 hours drip. Spray 2 days prior to fertilizing schedule (FNCA guidelines).

Purpose

Enhancing growth, increasing yield and reducing disease infection.

Application on vegetables farming

Dilute NM-OC to 100 ppm using water. Soak seeds for 2 hours and drip. Spray twice a week (FNCA guidelines).

Figure 3.0 Application of NM-Oligochitosan on chillies, paddy and vegetables farming.

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4. ISSUES, EFFECTS AND SOLUTIONS IN NM OLIGOCHITOSAN PRODUCTION

Table 1: Issues, effects and solutions in NM Oligochitosan production

ISSUES	EFFECTS	SOLUTIONS
RAW MATERIALS SPECIFICATIONS		
1. Each batch of chitosan received comes with various quality e.g high impurities, high insoluble substances.	❑ Inconsistent quality of final products. ❑ Chitosan exhibit low solubility property. ❑ Require process adjustment that involves a lot of time and energy ❑ Increase in production cost	❑ Request sample in advance for quality test prior to puchasing ❑ COA and MSDS required from the suppliers at every materials purchased. ❑ Chitosan specifications : i. Highly soluble in 1% acetic acid ii. DDA > 85% iii. Insolubles substances : < 1% ❑ Identify suppliers that provide chitosan with required specifications.
2. Low concentration of ethanol (< 30%)	❑ Shorten the shelf life of the final products (less than 2 years) ❑ Affect the quality of the final products	❑ Ethanol specifications i. industrial grade ethanol with 85 - 95% purity
3. Inappropriate acid for solubilizing chitosan	❑ Contribute to strong odour ❑ Affect the quality of the final products	❑ Suggest for only lactic acid.

4. Poor solubility of chitosan – chitosan with low solubility property consumes more than 24-mixing hours.	❑ Consume longer time and more energy. ❑ Increase in cost of production. ❑ Affect the quality of the final products	❑ Know-what chitosan properties required ❑ Purchase chitosan with DDA >85% ❑ Chitosan purchase n fine powder form instead of flake form.
ISSUES	**EFFECTS**	**SOLUTIONS**
IRRADIATION STAGE		
Molecular weight of irradiated chitosan out of allowed Mw range which is in the range of 10kDa – 20kDa.	❑ Oligochitosan does not function as PGP.	❑ Re-irradiate ❑ Dose optimization on chitosan to get desired Mw
Excessive packaging size of the chitosan supply.	❑ Difficult in handling during irradiation.	❑ Pakaging size should not exceed 25kg.
Packaging materials degrade upon high dose gamma radiation	❑ Unsuitable packaging materia l easily damages after irradiation. ❑ Packaging materials tend to degrade and contaminate the oligochitosan powder. ❑ Reduce quality of final product	❑ The use of radiation resistant packaging materials

*Irradiation facility is managed by Sinagama, customers should refer to Sinagama for further information on irradiation procedure.

ISSUES	EFFECT	SOLUTIONS
MIXING PROCESS		
Insufficient and unsuitable propellers . Current blade type is paddle type-blade	❑ Consume longer time for mixing process ❑ Oligochitosan powder tend to agglomerate than disperse homogeneously	❑ Identify the accurate types of propeller ❑ Propeller placed top and bottom side of the tank ❑ Suitable type of propeller (anchor blade – bottom side, blade paddles - top side) ❑ Production of minimum 750L.
Unsuitable chitosan open feeding system.	❑ Chitosan powder spills out during feeding process. ❑ Consume longer time and energy. ❑ Harmful to the personnels/workers. ❑ Affect the quality of final product.	❑ Continuous and close feeding channel ❑ Personnel/workers must apply PPE all time during production. ❑ Personnels/workers should take precaution whenever handling raw materials during production.
Unhomogeneous mixture	❑ Low quality of final product ❑ Longer mixing time	❑ Gradual increase in stirring rate ❑ Overnight stirring

ISSUES	EFFECT	SOLUTIONS
QUALITY PRODUCT MANAGEMENT		
Quality of final product reducedsbefore the expiry date	❑ Product deteriorated during storage due to storage condition. ❑ Sedimentation during storage. ❑ Pungent smell during storage.	❑ Storage of products at room temperature. ❑ Avoid from direct sunlight during storage.Shake well before use.
Unsuitable packaging type	❑ The quality product during storage is difficult to observe. ❑ Difficult to handle.	❑ The use of high density plastic screw cap bottle with stopper. ❑ Do not fill the cointainer in full in order to allow the expansion of liquid during storage (suggest to 10 - 15% of bottle remain with air). ❑ Avoid excessive air in product container in order to avoid product oxidation during storage.

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