UNIVERSITI TEKNOLOGI MARA

MEDIA ENHANCEMENT FOR BIOMASS OF EFFECTIVE MICROBES (E.M.) VIA AUTOMATED MEDIA OPTIMIZATION SYSTEM FOR BIOFERTILIZER FORMULATION

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of the requirements for the degree of
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(Applied Biology)

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CONFIRMATION BY PANEL OF EXAMINERS

I certify that a Panel of Examiners has met on 15 December 2021 to conduct the final examination of Aimi Nadia Binti Saharuddin on her **Master of Science** thesis entitled "Media Enhancement for Biomass of Effective Microbes (EM) Via Automated Media Optimization System for Biofertilizer Formulation" in accordance with Universiti Teknologi MARA Act 1976 (Akta 173). The Panel of Examiner recommends that the student be awarded the relevant degree. The Panel of Examiners was as follows:

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

The development of the agriculture sector acts as a base for the overall development of other sectors of the economy. It is vital to strategize ways to increase the production of agricultural products and improve soil vitality. The biofertilizer application is deemed today to limit the use of inorganic fertilizer and supports an effective tool for developing a green environment. The Effective Microbes (E.M.) biomass stock is in high demand as an additive in biofertilizer formulation as they improve soil-crops vitality and productivity. Nonetheless, the bio-productivity of the E.M. biomass stocks is unsurpassed by the demand due to non-optimized media and the use of the one factor at a time (OFAT) method, which is a less effective method. Thus, adopting a strategic approach is needed to overcome the issue. This research aimed to develop an optimized economic E.M. media with high yield and cost-effectiveness. This research intended to identify three objectives that affect E.M. growth performance; i) key nutrients, ii) nutrient interactions and media formulation, and iii) E.M. reproducibility from microlitre scale to flask scale. The effective method of the study utilizes a high-throughput Automated Media Optimization System (AMOS) and 150 µL microwell bioreactor based on a 96-microwell-plate). Three experimental phases aligned to the objectives are; phase 1 examined for vital nutrient screening, and phase 2 screened for the other micro and macronutrients (a total of 180 formulations) to formulate optimized media for E.M. biomass growth enhancement. The media optimization system utilized the Box-Behnken Design. To analyze the experimented data resulting from the screening, response-surface methodology (Main Effects and Interaction Effects) was applied to determine the formulations' key nutrients and nutrient interactions using Minitab 18 software. In Phase 3, the optimal media derived from the previous experiments were subjected to large-scale (1 L flask) reproducibility tests. In key nutrients screening, highest growth performance of EM was achieved in the supplemented media, for *B. subtilis* (0.07248 h^{-1}, 6.0 g/L YE + 40.0 mM G), *C. utilis*, (0.0795 h^{-1}, 12 mM $(NH_4)_2SO_4$ + 42 mM G), *P. acidilactici* (0.18433 h^{-1}, 10 g/L BE + 0.125 mM Mn), *R. palustris* (0.14557 h^{-1}, 7.2 mM YE + 40 mM SB), and *B. borstelensis* (0.102 h^{-1}, 4.0 g/L YE + 10 mM SB). For the nutrient interaction screening, the highest growth rate was attained at hour 2, and the doubling time was calculated as the followings – *B. subtilis* (2.782 h^{-1}, 0.42 h), *C. utilis* (2.3175 h^{-1}, 0.44 h), *P. acidilactici* (0.3831 h^{-1}, 0.62 h), *R. palustris* (1.2029 h^{-1}, 0.56 h), and *B. borstelensis* (0.9272 h^{-1}, 0.62 h). The nutrients' Main and Interaction Effects analysis was subjected to the p-value. The other effective microbes exhibited significant main effects except *P. acidilactici* and *B. borstelensis.* The pairwise nutrient interactions were also observed for all effective microbes. High biomass yields were obtained for the reproducibility test in the low rate media - 1.384 g/L for *B. subtilis*, 0.030 g/L for *P. acidilactici*, 0.388 g/L for *R. palustris*, and 1.563 g/L for *B. borstelensis*, whereas for *C. utilis* yielded 0.188 g/L in high rate medium. This research allows the understanding of nutrient interactions that control the induction of high growth performance and specific novel functions of various E.M. Also, novel and optimized media formulations were generated. The results obtained from this research, coupled with the effective utilization of AMOS, can greatly benefit the agriculture sector specifically, consumers and the community in general. In the future, more accomplishments can be achieved as compared to the current conventional practices.

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Aimi Nadia binti Saharuddin

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