BIODEGRADATION OF EXPANDED AND EXTRUDED POLYSTYRENES BY USING *Zophobas morio* LARVAE

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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DEDICATION

This thesis is dedicated to
All the People
who give me inspirations, support and encouragement

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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July 2022

Chair : Tan Geok Hun, PhD
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Malaysia faces a significant challenge in managing plastic trash, particularly non-recycled or illegally disposed of plastic wastes. Polystyrene (PS) is marked with a plastic resin number code of 6, which denotes that it is recyclable, but less recycled due to the costs and complicated recycling procedure. PS garbage pollutes the environment and poses a significant health risk to humans, animals and the marine ecology. PS consists of expanded (EPS) and extruded (XPS). EPS has a low density and low water diffusion resistance while XPS has a high density and high-water diffusion resistance. A recent study found that *Zophobas morio* larvae can be acts as a potential biodegradation agent in degrading PS wastes. Thus, first objective is to determine the growth development of *Z. morio* under controlled lab conditions. It was discovered that *Z. morio* go through a complete metamorphosis with four stages of growth: egg, larvae, pupa, and adult. Most importantly, it was noticed that larval stages can growth up to 190 ± 6 days. Larval stages are undergoing early instar, mid instar and late instar consists of 18 larval instars end up with 35.91 ± 4.12 mm larvae length, 3.88 ± 0.41 mm head capsule width and weight of 369.0 ± 81.9 mg. The growth development of other stages was also recorded to utilize it as a guideline for commercial production. A study showed that supplement added in larvae diets could enhance EPS and XPS degradation. Thus, second objective is to evaluate the effectiveness of degradation on EPS and XPS with different supplements diets using *Z. morio* larvae. The highest EPS and XPS consumption were found in oatmeal diets with 422.30 ± 2.44 mg and 268.33 ± 11.08 mg respectively. A molecular weight (Mw and Mn) was identified through GPC indicated that EPS and XPS molecular weight were reduced. ^{1}H NMR and FTIR analyses further proven depolymerization and partial oxidation of EPS and XPS occurred in all feeding diets. In overall, the efficiency of supplement on EPS and XPS degradation with ascending order such as cornmeal, wheat bran, oatmeal accordingly. Whilst, the following objectives is to investigate the growth development of *Z. morio* larvae after consuming expanded and extruded polystyrene with different diets. Results showed that larvae length (42.44 ± 2.50 mm), number of instars (17 ± 1 instars) and duration of larval (157 ± 6 days) was found in oatmeal as a supplement diet contains EPS foam which is opposed to control diets. Same

phenomena were occurred in XPS foam diets. This indicate that sufficient nutrient provided may enhance larvae growth, reduce larval instar number and shorten larval development period. Lastly, oatmeal as a supplement diet with EPS and XPS were used to study microbial population in the gut system of *Z. morio* larvae. Analysis of gut microbiome by next-generation sequencing revealed that *Kluyvera spp*., *Klebsiella spp*., and *Enterobacter spp*. was showed closely associated in EPS and XPS degradation. Supplements added enhance larvae growth and assisting larvae degrade EPS and XPS wastes.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

BIODEGRADASI POLISTIRENA BERKEMBANG DAN TERSEMPERIT DENGAN MENGGUNAKAN LARVA *Zophobas morio*

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Malaysia menghadapi cabaran yang besar dalam menguruskan sampah plastik, terutamanya pembuangan plastik yang tidak dikitar semula atau dilupuskan secara haram. Polistirena (PS) ditandakan dengan kod nombor resin plastik 6, yang menunjukkan bahawa ia boleh dikitar semula, tetapi kurang dikitar semula disebabkan kos dan prosedur kitar semula yang rumit. Sampah PS telah mencemarkan alam sekitar dan menimbulkan risiko kesihatan yang ketara kepada manusia, haiwan dan ekologi marin. PS terdiri daripada polistirena berkembang (EPS) dan polistirena tersemperit (XPS). EPS mempunyai ketumpatan rendah dan rintangan resapan air yang rendah manakala XPS mempunyai ketumpatan tinggi dan rintangan resapan air tinggi. Kajian terbaru mendapati larva *Zophobas morio* boleh bertindak sebagai agen biodegradasi yang berpotensi bagi pembuangan PS untuk degradasi. Oleh itu, objektif pertama adalah untuk menentukan perkembangan pertumbuhan *Z. morio* di bawah keadaan makmal terkawal. Hasil kajian didapati bahawa *Z. morio* melalui metamorfosis lengkap dengan empat peringkat pertumbuhan: telur, larva, pupa, dan dewasa. Paling penting, diperhatikan bahawa peringkat larva boleh bertumbuh sehingga 190 ± 6 hari. Peringkat larva melalui proses instar awal, instar pertengahan dan instar akhir terdiri daripada 18 instar larva berakhir dengan panjang larva 35.91 ± 4.12 mm, lebar kapsul kepala 3.88 ± 0.41 mm dan berat 369.0 ± 81.9 mg. Perkembangan pertumbuhan peringkat lain juga direkodkan untuk digunakan sebagai garis panduan pengeluaran komersial. Satu kajian menunjukkan bahawa suplemen yang ditambah dalam diet larva boleh meningkatkan degradasi EPS dan XPS. Oleh itu, objektif kedua adalah untuk menilai keberkesanan degradasi pada EPS dan XPS dengan diet suplemen berbeza menggunakan larva *Z. morio*. Pengambilan makanan EPS dan XPS tertinggi didapati dalam diet oatmeal dengan 422.30 ± 2.44 mg dan 268.33 ± 11.08 mg masing-masing. Berat molekul (Mw dan Mn) telah dikenal pasti melalui GPC menunjukkan bahawa berat molekul EPS dan XPS telah dikurangkan. Analisis ^{1}H NMR dan FTIR telah mengenalpasti penyahpolimeran dan pengoksidaan separa EPS dan XPS berlaku dalam semua diet. Secara keseluruhannya, kecekapan suplemen pada degradasi EPS dan XPS dengan susunan menaik seperti tepung jagung, dedak gandum, oatmeal dengan sewajarnya. Manakala, objektif berikut adalah untuk mengkaji perkembangan pertumbuhan larva *Z. morio* selepas pengambilan polistirena bergembang dan tersemperit dengan diet yang berbeza. Keputusan menunjukkan bahawa panjang larva (42.44 ± 2.50 mm), bilangan instar (17 ± 1 instar) dan tempoh larva (157 ± 6 hari) didapati dalam oatmeal sebagai diet tambahan mengandungi EPS yang bertentangan dengan diet kawalan. Fenomena yang sama berlaku dalam diet XPS. Ini menunjukkan bahawa nutrien yang mencukupi boleh meningkatkan pertumbuhan larva, mengurangkan bilangan instar larva dan memendekkan tempoh perkembangan larva. Akhir sekali, oatmeal sebagai diet suplemen dengan EPS dan XPS digunakan untuk mengkaji populasi mikrob dalam sistem usus larva *Z. morio*. Analisis mikrobiom usus oleh penjujukan generasi akan datang mendedahkan bahawa *Kluyvera spp*., *Klebsiella spp*., dan *Enterobacter spp.* telah menunjukkan berkait rapat dalam degradasi EPS dan XPS. Penggunaan suplemen telah meningkatkan pertumbuhan larva dan membantu larva dalam degradasi pembuangan EPS dan XPS.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF SYMBOLS AND ABBREVIATIONS

%	Percentage
=	Equal
^{0}F	Degree Fahrenheit
°C	Degree Celcius
w/v	Weight per volume
ANOVA	Analysis of variance
CB	Corn bran
cm	Centimetre
CRD	Completely randomized design
CTAB	Centrimonium bromide
DNA	Deoxyribonucleic Acid
ds	Double-strand
E	East
e.g	Example
EDTA	Ethylenediamine tetraacetic acid
EPS	Expanded polystyrene
FAO	Food and Agriculture Organization
g	gram
GDP	Gross Domestic Product
h	Hour
HCl	Hydrochloric acid
HDPE	High-density polyethylene
kg	kilogram
LDPE	Low-density polyethylene

M	Molar
m	meter
mg	Miligram
min	minute
mL	Millilitre
mM	Millimolar
mm	Millimetre
MSW	Municipal solid waste
N	North
NaCl	Sodium chloride
NaH_2Po_4	Monosodium hydrogen phosphate
nm	Nanometers
OB	Oatmeal
OTU	Operational taxonomic units
PET	Polyethylene terephthalate
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinyl chloride
RH	Relative humidity
RNA	Ribonucleic Acid
rpm	Revolutions per minute
SDS	Sodium dodecyl sulfate
sec	Second
Sp	Particular species
TAE	Tris-acetate-EDTA

tons	tonnes
WB	Wheat bran
XPS	Excluded polystyrene

CHAPTER 1

INTRODUCTION

Plastic pollution has been a worldwide environmental problem for a long time (Smith & Brisman, 2021). The seeming disparity between the extraordinary durability of plastics and their limited-service life results in a rising buildup of plastic trash in the environment. (Yang *et al*., 2018a). Plastic production has continued to swiftly slant upward and begun to double since commercial manufacturing began in the 1950s, (Ryan, 2015; Chris *et al*., 2015). A corresponding increase has followed this growth in output in the marine environment's plastic concentration (Alabi *et al*., 2019). Plastic's durability means that if it is not subjected to microorganisms, UV radiation, or other conditions, it may persist for years to millennia (Webb *et al*., 2013). Plastic particles have been discovered in the world's oceans accumulated up to 580,000 pieces per square kilometre (Amelia *et al.*, 2021; Chris *et al*., 2015). Besides that, plastic waste was threatened wildlife and indirectly affected human health (Thushari & Senevirathna, 2020; Thompson *et al.*, 2009)**.**

The plastics manufacturing industry grew at the fastest pace since the year 2000 when compared to other sectors in Malaysia (Chen *et al*., 2021). Malaysia is becoming one of the top plastic manufacturing industries with about 1300 plastic industries (Chen *et al*., 2021; MESTECC, 2018). However, Malaysia has severe plastic waste management problems, especially those not recycled or properly disposed of plastic waste. Malaysia ranked 8th worst plastic waste management among the top ten countries globally (MESTECC, 2018). A total of 0.94 million tons of unmanaged plastic trash output where 0.14-0.37 million tons ended up in the seas, poisoning and suffocating marine life including seabirds (Jambeck *et al.*, 2015). Although several recycling campaigns were introduced but over the years have had too little impact.

Polystyrene is a frequently used plastic polymer [-CH (C_6H_5) CH_2-]-. The annual polystyrene production rate surpasses 20 million tons per year (Yang *et al*., 2018b). Polystyrene wastes are a recalcitrant plastic polymer and it is among one of the widespread human-made plastic pollutants. It is a form of polystyrene oil-based product (Khalid *et al.*, 2012). Polystyrene consists of expanded (EPS) and extruded types (XPS). Both are made from styrene polymer but the density, cell structure and production method are different. Disposable food service wares such as plates, cups, food containers, cartons, trays and bowls are often made of polystyrene (Nukmal *et al.*, 2017). Hawker stalls are likely to use for food packages as their light weight and colourless give customers a sterile and hygiene appearance. Besides, hypermarkets used polystyrene for fruit and vegetable packaging to reduce compression, impact, abrasion, tension, vibration, bending and shear. It is resistant to acid and salt, high water and vapour resistance, which can be repeatedly used for longer (Dubey & Mishrad, 2018). Polystyrene is labelled with a plastic resin code of 6, indicating recyclable (The Star, 2017; NSWMD, 2011), but less plastic foam is recycled due to the high cost and miscellaneous recycling process (Stewart, 2019). Thus, the accumulation of plastic foam waste led to landfills and litter elsewhere. Besides, polystyrene waste polluted the environment and brought severe health problems to humans, wildlife, and marine ecosystems (Setyowati, 2014). Polystyrene foam debris left in the environment is easily ingested by wildlife (Schwade *et al.*, 2016) and marine life (Davidson, 2012) mistakenly as a food source cause life-threatening. Reactive metabolites of styrene named styrene oxide and other carcinogenic substances on polystyrene such as formaldehyde and benzene cause human health problems (Nukmal *et al.*, 2017; Setyowati, 2014). Abiotic techniques with incineration may be used to reduce polystyrene wastes, yet such operations emit harmful compounds into the air, pollute the environment, and produce dangerous by-products (Yang *et al*., 2018a). Therefore, a cost-effective and long-term solution should concentrate on source control and cleanup. Biodegradation should be focus on polystyrene waste recycling. Biodegradation of plastic is a preponderance of biological activity in depolymerising plastic polymers into lower molecular weight molecules and mineralising into carbon dioxide and water (Mohanan *et al*., 2020).

1.1 Problem statement

Malaysia generated 19% plastic waste in overall waste production. The plastic waste contained single-use plastics and foam plastic with 17% and 9%, respectively (Chen *et al.*, 2021). In Malaysia, takeaway packaging and carrying cartons made of polystyrene are seldom recycled due to the plastic waste is fragile, low-quality and tainted with food residue (The Sun, 2021). The polystyrene waste ends up in landfills, incineration and litter elsewhere. Biodegradation and bioremediation have been emphasized against plastic waste for years (Ali *et al.*, 2021; Sharma *et al*., 2017). In Malaysia, a farm located in Kuching, Sarawak utilized maggots and superworm (*Zophobas morio* larvae) to digest food waste collected from restaurant and hawker stalls (New Straits Times, 2017). Surprisingly, *Z. morio* larvae with rapid food waste consumption and its healthy growth has gain interesting to the researcher. *Z. morio* larvae are commonly utilized as animal, insect and aquaculture feed due to high protein content (Rumbos & Athanassiou, 2021; Jabir *et al.*, 2012). Tang *et al.* (2017) study revealed that *Z. morio* larvae could degrade various plastic materials. Thus, it can be used for further studies on polystyrene waste degradation purposes. Yang *et al*. (2020) results showed that *Z. morio* larvae ingested 0.58 mg/day of polystyrene as a single diet within 28 days which is four times higher than *T. molitor* larvae. Thus, a better understand on *Z. morio* life cycle could help Malaysia to solve the polystyrene waste management problem in a sustainable practice. Currently, life cycle information on *Z. morio* is still unclear and uncertainty due to variation in biotic and abiotic factors. Besides, varied diets or food supplements provided may play an important role in assisting *Z. morio* larvae to enhance polystyrene consumption and degradation but lack of supported information as researcher study focus more on polystyrene as a sole-diets. Furthermore, it is believed that food supplements may regulates the gut community to degrade polystyrene. These potential microbes can be used for future isolation and enzyme discovered in enhance polystyrene degradation.

1.2 Research Objectives

The purpose of the current investigation is to identify potential additives contributions made on *Z. morio* larvae in assisting degradation of EPS and XPS plastic wastes. To accomplish the goal, the following objectives are created:

1. To determine the growth development of *Z. morio* under controlled lab condition

2. To evaluate the effectiveness of degradation on expanded polystyrene and extruded polystyrene with different supplements diets using *Z. morio* larvae

3. To investigate the growth development of *Z. morio* larvae after consuming expanded and extruded polystyrene with different diets

4. To identify the microbe population in the gut system of *Z. morio* larvae among treatment with high polystyrene (XPS and EPS) consumption

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