IMPROVEMENT OF VEGETABLE WASTE COMPOSTING PERFORMANCE BY THE ADDITION OF HYDROLASE-PRODUCING BACTERIA

By

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Large quantities of organic waste are produced in modern society and its disposal poses serious environmental and social problems. Malaysia recorded a total volume of 16 metric kilotons per day in food waste. Given this situation, it is crucial to research how food waste degrades, especially vegetable waste, which accounts for nearly half of all food waste produced and poses a serious risk to the environment due to the release of gases and pollutants as well as the potential for infection. This study aimed to isolate hydrolase-producing bacteria (HPB) to improve biodegradation of vegetable waste. A total of 26 bacterial strains were tested through isolation from fermented vegetables, agricultural soils, and those from our glycerol stock collections. Two strains identified as Bacillus licheniformis 2D55 and Bacillus xiamenensis Y7 exhibited activities of multi-hydrolytic enzymes which are amylase, protease, lipase, and cellulase. An in-vitro biodegradation kitchen waste was done through solid-state fermentation (SSF). Co-culture fermentation which consists of rice, vegetable waste and both strains selected demonstrated synergistically improved hydrolytic activities during SSF. The hydrolase activities, microbial count, percentage of biodegraded total solids and gross degradation rate in co-culture treatments were observed superior to the respective mono-cultures and uninoculated control. The co-culture treatments had the highest proportion of biodegraded total solids in sterile SSF (54.73%) and non-sterile SSF (65%) as well as the highest gross degradation rate percentage in sterile SSF (25.39%) and non-sterile SSF (41.36%) after the 14-day fermentation process. The composting process was done in two months and various parameters (moisture content, pH, temperature, microbial count, carbon to nitrogen (C/N) ratio and percentage of biodegraded total solids) were observed and maintained throughout the experiment. In the composting experiment, the inoculated compost was observed superior to the control compost as inoculated recorded higher hydrolase activities and microbial count. In addition, inoculated compost also had higher proportion of biodegraded total solids (63.02%) compared to control (29.80%). Inoculated compost achieved slightly higher temperature during the early stage of composting and with foul odour reduced compared to control. The final inoculated compost was well-matured after two months with germination index, GI (>100%), pH value of around 7, C/N ratio (< 20), dark colour and earthy smell. The overall results demonstrated that inoculating HPB co-cultures not only able to improve vegetable waste biodegradation but also enhancing germination and radical growth.

Keywords: Hydrolase-producing Bacteria, Biodegradation, Solid-state Fermentation, Composting, Kitchen Waste

SDG: Goal 12: Responsible Consumption and Production

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PENINGKATAN PRESTASI PENGKOMPOSAN SISA SAYURAN DENGAN PENAMBAHAN BAKTERIA PENGHASIL HIDROLASE

Oleh

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Kuantiti sisa organik yang banyak terhasil dalam masyarakat moden dan pembuangannya menimbulkan masalah sosial dan alam sekitar yang serius. Malaysia mencatatkan jumlah keseluruhan 16 kiloton metrik sehari dalam sisa makanan. Melihat kepada keadaan ini, adalah penting untuk menyelidik bagaimana untuk membuang sisa makanan, terutamanya sisa sayuran, yang menyumbang hampir separuh daripada semua sisa makanan yang dihasilkan dan perkara ini menimbulkan risiko serius kepada alam sekitar akibat daripada pembebasan gas dan bahan pencemar yang berpotensi menyebabkan jangkitan. Penguraian biologi dianggap sebagai pendekatan penting dalam menyelesaikan isu berkaitan sisa buangan kerana ia memberi tumpuan kepada peranan rembesan enzim hidrolitik melalui aktiviti mikrob yang ditambah dan/atau aktiviti mikrob semula jadi. Kajian ini bertujuan untuk memencilkan bakteria penghasil hidrolase (HPB) bagi meningkatkan penguraian secara biologi sisa sayuran. Sebanyak 26 bakteria telah diuji melalui pemencilan daripada sayur-sayuran yang difermentasi, tanah pertanian, dan daripada koleksi stok gliserol kami. Dua bakteria yang dikenal pasti sebagai Bacillus licheniformis 2D55 dan Bacillus xiamenensis Y7 mempamerkan aktiviti enzim multi-hidrolitik iaitu amilase, protease, lipase, dan selulase. Penguraian biologi sisa sayur in-vitro dilakukan melalui fermentasi pepejal (SSF). Fermentasi kultur bersama melibatkan nasi, sisa sayur dan dua bakteria yang telah dipilih menunjukkan aktiviti hidrolitik secara sinergistik dapat dipertingkatkan semasa SSF. Aktiviti hidrolase, kiraan mikrob, peratusan jumlah pepejal terurai secara biologi dan kadar penguraian kasar dalam rawatan kultur bersama diperhatikan lebih tinggi daripada monokultur dan tanpa inokulasi bakteria. Rawatan bersama kultur mempunyai nilai peratusan tertinggi jumlah pepejal terurai secara biologi dalam SSF steril (54.73%) dan SSF tidak steril (65%) serta peratusan kadar penguraian kasar tertinggi dalam SSF steril (25.39%) dan SSF tidak steril (41.36%) selepas proses fermentasi selama 14 hari. Proses pengkomposan dilakukan dalam dua bulan dan pelbagai parameter (kandungan lembapan, pH, suhu, kiraan mikrob, nisbah karbon kepada nitrogen (C/N) dan peratusan jumlah pepejal terurai secara biologi) diperhatikan dan dijaga sepanjang eksperimen. Dalam eksperimen pengkomposan, kompos dengan inokulasi diperhatikan lebih baik daripada kompos tanpa inokulasi kerana kompos dengan inokulasi merekodkan aktiviti hidrolase dan kiraan mikrob yang lebih tinggi. Di samping itu, kompos dengan inokulasi juga mempunyai nilai peratusan yang lebih tinggi bagi jumlah pepejal terurai secara biologi (63.02%) berbanding kompos tanpa inokulasi (29.80%). Kompos dengan inokulasi mencapai suhu yang lebih tinggi sedikit semasa peringkat awal pengkomposan dan dengan pengurangan bau busuk berbanding kompos tanpa inokulasi. Kompos dengan inokulasi pada pengakhiran dua bulan telah matang dengan indeks percambahan, GI (>100%), nilai pH sekitar 7, nisbah C/N (< 20), warna gelap dan berbau tanah. Keputusan keseluruhan menunjukkan bahawa inokulasi bersama HPB bukan sahaja dapat meningkatkan penguraian secara biologi sisa sayuran tetapi juga meningkatkan percambahan dan pertumbuhan radikal.

Kata Kunci: Bakteria Penghasil Hidrolase, Biodegradasi, Fermentasi Pepejal, Pengkomposan, Sisa Dapur

SDG: Matlamat 12: Penggunaan dan Pengeluaran Bertanggungjawab

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LIST OF ABBREVIATIONS

%	Percentage
$^{\circ}C$	Degree celsius
kg	Kilogram
N_2O	Nitrous oxide
µL	Microliter
µm	Micrometer
µg	Microgram
CFU/mL	Colony forming unit per mililitre
$CaCl_2$	Calcium chloride
NH_3	Ammonia
CO_2	Carbon dioxide
g	Gram
NaCl	Sodium chloride
$(NH_4)_2SO_4$	Ammonium sulphae
KH_2PO_4	Monopotassium phosphate
CMC	Carboxymethylcellulase
M	Molarity
MRS	de Man Rogosa Sharpe broth and agar
mg	Milligram
mL	Millilitre
mM	Millimolar
N	Normality
$MnSO_4$	Manganese sulphate
Na_2CO_3	Sodium carbonate

p-NPP	p-nitrophenypalmitate
nm	Nanometer
OD	Optical Density
g/L	Gram per litre
cm	Centimetre
YEPD	Yeast extract potato dextrose
rpm	Revolutions per minute
CH_4	Methane
U/mL	Unit per mililitre
v/v	Volume per volume
w/v	Weight per volume

CHAPTER 1

INTRODUCTION

The waste generated by commercial, domestic, and institutional operations is known as municipal solid waste (MSW). On a global scale, landfilling is the most popular disposal method in MSW management (Townsend et al., 2015). MSW management has become a difficult task in Malaysia, owing to rising greenhouse gas emissions (e.g., methane gas) from the decomposition of organic waste in landfills, as well as the demand for more energy (electricity) and material consumption to sustain the country's growing population (Yong et al., 2019). Currently, 89% of generated MSW is dumped with inadequate treatment, and only 1% of overall inbound MSW is treated properly (Chien Bong et al., 2017). Based on the reports, the major MSW fractions generated in Malaysia include 45% organic material (food waste), 13% plastics, 12% diapers, 9% paper, 3% glass, 3% metal and others (Zaipul & Ahmad, 2017). Therefore, one of the primary issues in the MSW management process in Malaysia is dealing with organic waste or food waste.

In 2018, food waste in Malaysia recorded a total volume of 16 metric kilotons daily. According to statistics, a volume of 20-50% of fruits and vegetable wastes are disposed along the production chain in 2018 as a result of poor management (Then et al., 2021). The non-edible portions of vegetables that are thrown during handling, collecting, processing and transportation are known as vegetable waste (Chang et al., 2006). In this context, it is important to study the degradation process of food waste especially vegetable waste as it contributes to almost half of the total food waste generated causing serious environmental issues because of the discharge of gases pollutants and possibility for infections to spread (Lu et al., 2022). Due to the nature of vegetable waste and the required equipment to treat them, treatment like animal feeding, anaerobic digestion, and incineration are not always practical alternatives (Sarkar et al., 2016). Major treatment used in managing the MSW involving incineration and landfilling. Although these methods are more effective in reducing large amount of MSW, they can cause harm to the environment in the long run by producing dangerous gases, bad odours and harmful greenhouse gases. Therefore, another alternative method that can be used to treat vegetable waste are solid-state fermentation (SSF) and composting. These approaches are more adaptable, comparatively faster and reliable to handle with the daily vegetable produced.

SSF has various benefits over traditional submerged fermentation, including product stability, reduced pollutant emission, high fermentation productivity, and product end-concentration (El-Bakry et al., 2015). SSF can also use inexpensive and renewable resources such waste molasses, sewage sludge, agricultural and forestry wastes, food waste, and animal manure (Zhang et al., 2015). Another method that can be used is composting. Composting is the most preferred, eco-friendly, and economically viable waste treatment process when properly handled (Storino et al., 2016). There are numerous environmental advantages to composting organic waste instead of dumping it in landfills. For instance, soil properties such as texture, porosity, and organic matter are improved, and landfill greenhouse gas emissions (GHGs) are reduced (Rastogi et al., 2020).

Microbial enzymes have received interest for their worldwide usage in medical sector and various other industries (e.g., food, agriculture, chemicals and medications) due to their stability, catalytic performance and simplicity of manufacture and optimization. The use of enzymes in these industries is rapidly increasing due to their short processing time, low energy input, cost effectiveness, non-toxic, and biodegradable features. Microbial enzymes can degrade or convert chemical substances e.g., organic wastes, phenolic compounds, nitriles, amines, etc. found in industrial and domestic wastes (Singh et al., 2016). Hydrolases such as amylase, protease, lipase, cellulase are omnipresent and widely distributed among microorganisms, in particular within bacteria and fungi (Drissi Kaitouni et al., 2020). Since the most common components in composting comprises a complex mix of organic matters such as vegetable waste, cereal straws, different types of crop husks and various types of manure (Gaind, 2014; Sánchez et al., 2017), hydrolase-producing bacteria (HPB) might be a great tool to treat vegetable waste efficiently.

Vegetable waste composting components usually consists of crop straw, animal manure and vegetable waste (Wan et al., 2020). Various studies have been conducted related to vegetable waste composting. However, only few studies on substance conversion in vegetable waste have been reported, including the discussion on the effect of microbial inoculation in the biodegradation of vegetable waste. Studies on the development of effective hydrolytic strains for vegetable waste breakdown and the selection of competitive microbial agents are limited. Biomass reduction criteria serve as the primary goal and best indicator of vegetable waste biodegradation performance. Therefore, this research aimed to enhance vegetable waste biodegradation by using the isolated HPB as an inoculant for commercial uses in waste biodegradation, such as SSF and composting. Initially, by in-vitro biodegradation of kitchen waste which comprised of vegetable waste and rice in lab-scale SSF, the ability of selected strains to break down kitchen waste was defined based on hydrolase activities and waste biomass reduction. Finally, the effects of the HPB on the composting of vegetable waste was evaluated.

This study embarks on the following specific objectives:

1. To isolate, screen and identify hydrolase producing microorganisms for biodegradation process.
2. To examine the in-vitro kitchen waste in solid-state fermentation and vegetable waste composting process by addition of the hydrolase producing microorganisms.
3. To evaluate vegetable waste composting quality by conducting maturity test.

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