PRODUCTION OF VALUABLE ENZYMES THROUGH FERMENTATION OF TROPICAL FRUIT DREGS

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TO MY BELOVED FAMILY AND SSN

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ABSTRACT

The amount of waste in Malaysia is getting higher due to the increasing of population and development. The statistic showed that Malaysian generated 23,000 tonnes of waste in the year 2008 and the amount is expected to rise until 30,000 tonnes by the year 2020. From the waste generated, there is about 48% of organic and food waste. Therefore, it has been many solutions suggested nowadays to solve the organic waste management problem, especially in the conversion of waste into value added products. In this study, a bioconversion of selected tropical fruit dregs into valuable enzymes was investigated. The tropical fruit dregs used in the fermentation were papaya, pineapple and guava. The optimization of amylase activity, lipase activity, reducing sugar concentration and biomass content were carried out for 120th day of the tropical fruit dregs fermentation by response surface methodology. The fermentation of guava waste performed an optimum amylase activity at 0.05 U/ mg whereas the fermentation of papaya, pineapple and guava waste showed an optimum lipase activity at 111.33 U/mL. For the optimization of multiple responses, the combination of papaya, pineapple and guava waste performed the optimum enzyme activities at 95th day of the fermentation period. The optimum amylase and lipase activities were 0.0289 U/mg and 105.971 U/mL respectively. These valuable enzymes that produced during the fermentation process are believed to be a good cleaning agent.

ABSTRAK

Jumlah sisa di Malaysia semakin meningkat disebabkan oleh peningkatan bilangan penduduk dan pembangunan negara. Statistik menunjukkan Malaysia telah menjana 23,000 tan sisa buangan pada tahun 2008 dan jumlah itu dijangka akan meningkat sehingga 30,000 tan metrik menjelang tahun 2020. Sebanyak 48% daripada sisa buangan itu adalah terdiri daripada sisa organik dan makanan . Oleh itu, banyak cara penyelesaian telah disyorkan pada hari ini untuk menyelesaikan masalah pengurusan bahan buangan organik, terutamanya dengan cara menambah nilai pada sisa buangan sehingan menjadi produk nilai tambah. Proses penukaran dan penambahan nilai dari sesetengah buah-buahan tropika yang dipilih kepada enzim yang berguna akan dikaji dalam kajian ini. Sisa buah-buahan tropika yang digunakan dalam panapaian ialah betik, nanas dan jambu. Satu analisis tentang mengoptimumkan ujian enzim pada assay amilase dan lipase serta kepekatan gula dan kandungan biomas telah dijalankan sepanjang 120 hari dalam tempoh proses penapaian dengan menggunakan kaedah gerak balas permukaan. Penapaian sisa guava menunjukkan aktiviti amylase yang optimum pada 0.05 U/mg manakala penapaian sisa gabungan betik, nanas dan jambu menunjuakkan aktiviti lipase yang optimum pada 111.33 U/mL. Bagi pengoptimuman pelbagai factor, sisa gabungan betik, nanas dan jambu telah menghasilkan enzim secara optimum pada hari ke-90 dalam tempoh proses penapaian. Aktiviti amilase dan lipase yang optimum adalah 0,0289 U / mg dan 105,971 U / mL masing-masing. Enzim bernilai yang dihasilkan semasa proses penapaian dipercayai dapat menjadi sebagai ejen pembersihan yang baik.

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LIST OF SYMBOLS

˚C	Degree Celcius
%	Percentage
w/v	Weight/Volume
v/v	Volume/Volume
g	Gram
mL	Milliliter
µL	Microliter
cm	Centimeter
M	Molar
mM	Millimolar
µg/mL	Microgram per milliliter
rpm	Revolutions per minute
Ve	Elution volume
Vo	Void volume
kDa	Kilo Dalton
U/mL	Unit of lipase per milliliter

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The issue of the environmental quality is enthusiastic in these recent years (Li *et al*., 2009). These may related to the continuous development of the countries in order to improve the lives of citizens. The development is either directly or indirectly affected the environment which causes climate change and global warming to the earth. As the residents on this planet, human beings are suffering from the adverse effects of the development instead of enjoying the life. Therefore, many countries started to promote the concept of sustainable development. With this concept, all the processes of development are assurance for not destroying the environment or ecological balance.

There are many factors that contribute to the degradation of environmental quality. Among these factors, the management of household and industrial waste is the most serious issue (Jalil, 2010). In Malaysia, an average of 3500 tonnes of wastes has been generated by the citizens per day (Tarmudi *et al*., 2009). All the wastes will end up as the landfill and around 50% of the wastes are organic wastes (Behzad *et al*., 2011). Landfill is traditional waste management in Malaysia. There are about 230 operating landfill sites in Malaysia, however, majority of the landfill are poorly managed (Masirin *et al*., 2008). The situation is definitely against the concept of sustainable development and further brings down the development of a country.

Many researches focused on the study of organic wastes as it occupies the large portion of the total wastes. Generally, organic wastes are anything that comes from biological origins that are biodegradable, such as fruits, vegetables, meats, crop wastes and so on. As the population keeps rising, the rate of producing organic wastes is also getting increase. Inadequate technologies in managing the large amount of wastes cause severe pollution to the environment. In this situation, the government is not the only one to blame on, as the residents should also pay the responsibility on the problems.

Tan (2009) had introduced the simple fermentation method of organic wastes to the world. With this simple fermentation of organic wastes, everyone can make their wastes into useful products (garbage enzymes) and not simply dispose the wastes into landfills or bring into the incinerators. The raw materials that used in fermentation are organic waste such as vegetables and fruit dregs, sugar and water (Bhavani, 2011). The fermentation process is considered as anaerobic process as there is no oxygen supply to the medium and the container will be closed tightly throughout the fermentation process.

Enzymes will be produced throughout the fermentation process, which is termed as “garbage enzyme” (Tan, 2009). The garbage enzymes are believed to act as cleaning agents, fertilizer and even also insecticide. In general, enzymes also well-known as biocatalysts which are important in living organisms as it can increase the rate of chemical reaction. Therefore, the production of garbage enzymes will indirectly replace non-biodegradable cleaning materials and responsible in decomposing toxic chemicals into harmless types.

Organic wastes such as fruit dregs can be obtained from every household as fruits are good source of vitamins, antioxidants, fibre and minerals that required by everyone on the Earth. Therefore, fruit dregs such as fruit peels, cuttings and bits are the common organic wastes that generated by us. If every household is able to utilize the simple fermentation method in fermenting fruit dregs at home, the effort will not only reduce the wastes that going to dispose in the landfills, but also save the environment.

In this study, tropical fruit dregs are being fermented using the simple fermentation method. The tropical fruit dregs that are going to use is from papaya, pineapple, and guava. The reason of choosing these tropical fruits is based on its availability in Malaysia. As theoretically, those fruits are basically from tropical country and Malaysia is one of the major exporters. Besides that, there is no season for the fruits to grow and the fruits can be obtained throughout the year. Second reason is that the demand of domestic food products is getting increase. The introduction of tropical fruits in the market is successful as it is safer to consume if compared to the imported fruits.

There is still no scientific research on the garbage enzymes that fermentated using simple fermentation method. Therefore, the reason of why the garbage enzymes are useful in cleaning purposes needs to be further investigated scientifically. Bhavani (2011) assumed that the end product which is useful in cleaning purpose is basically acetic acid which is known as non-toxic cleaner based on its acidic properties. Anyway, the garbage enzymes contributed with its low cost production, no addition of synthetic chemicals and production of high energy. If everyone can make simple fermentation of organic wastes instead of disposing or incinerating the wastes improperly, environmental pollution can be reduced.

1.2 Problem Statement

The increasing amount of organic wastes and the waste management system in Malaysia had always become an issue of the environmental problem. Many researches are done on bioconversion of the wastes into other valuable products rather than disposed the wastes into landfills. The bioconversion of organic wastes is typically referred to fermentation of the wastes that collected from industrial area. However, the environmental problem still cannot be solved as the organic wastes that produced by household are difficult to categorize and further process the wastes into valuable products.

Simple fermentation method is introduced where every household is able to put their responsibility on the organic wastes that had been generated. The fermentation will produce valuable enzymes which also term as “garbage enzyme”. Garbage enzyme is excellent in the cleaning activities as it is believed that enzymes are excreted after three months of fermentation (Tan, 2009). However, there is still lack of scientific evidence to prove the duration for the enzymes to be produced and the amount of enzyme that can be produced by using different raw materials in the fermentation process.

1.3 Objectives

The objective of this study is to optimize the enzyme production of fermented tropical fruit dregs by using response surface methodology.

1.4 Scope of Study

In this study, the selected tropical fruit dregs are fermented only with molasses and water. The selected tropical fruit dregs are included papaya, pineapple, and guava. The reasons of choosing these tropical fruit dregs are based on its availability in Malaysia and there are non-seasonal fruits. Molasses with the lower cost are being chosen instead of brown sugars. This is due to the higher potential of un-processing sugars as carbon source in the fermentation. Dark brown liquid with unpleasant smell will be produced after complete fermentation step. It is believed that microorganisms that present in the fruit dregs utilize molasses as their carbon source and further produce useful enzymes. These useful enzymes can be used as cleaning agent in the routine household activities.

The optimization of the tropical fruit dregs fermentation is performed by response surface methodology (RSM). RSM is useful in assembling a functional relationship between the response values obtained from experiments and a set of design variables. In this study, the design variables are the incubation time and sample varieties. There are seven fermentation samples that will be fermented with certain ratio of pineapple, papaya and guava wastes. Each of the fermentation samples will be tested on several enzyme activities at 30^{th}, 60^{th}, 80^{th}, 90^{th}, 100^{th}, $110^{th}$and 120^{th} days.

These enzyme activities included lipase and amylase. Lipase and amylase are chosen instead of protease because protease is the promising enzyme in cleaning purpose and many researches have been done on it. Study of lipase and amylase are under highly concern nowadays based on their potential of biodegradation in the environment. Besides that, these enzymes loss their enzymatic activity under washing and cleaning conditions which reduces pollution problems.

The residue mass of the sample and the concentration of reducing sugar (glucose) are also being tested besides the enzyme activities. These are to ensure the completion of the fermentation process. The residue mass of the sample and reducing sugar concentration are increased following the raising of enzyme activities.

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