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LIPASE PRODUCTION FROM SOLID STATE FERMENTATION OF COPRA WASTE ASSOCIATED FUNGUS *Aspergillus niger*

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Abstract: Lipases are enzyme with versatile industrial applications can be produced by the solid-state fermentation (SSF) method and is an economical alternative for enzyme production assisted by fungus. In Malaysia, 5 million of copra waste were generated annually. Large amount of copra waste produced will cause an increasing amount of the waste dumped to the landfill. Copra waste is one of the potential substrates to produce lipase enzyme through SSF. Thus, the aim of this study is to optimize the lipase production by SSF associated by *Aspergillus niger* using the 2^3 full factorial design approach. In this study the factors affecting parameters that involved in the production of lipase enzyme such as temperature (25˚ and 35˚), substrates concentration (40% and 60%) and inoculum size of *Aspergillus niger* (1 and 9 petri dish) were determined. The maximum production of lipase was obtained after 120-hour incubation in SSF. The optimum condition for inoculum size of *Aspergillus niger* was 9 plates, 30°C of incubation temperature and 60 % moisture contents. The range of the concentration of lipase enzyme produced varied from 105 U/ml to 170 U/ml. When applied to the wastewater treatment, the reducing percentage of fat, oil and grease (FOG) in food processing wastewater is reduced from 219.4925mg/l to 169.467mg/l accounted to the amount of 34 % FOG removal. Lipase produced using copra waste as a substrate using SSF has the potential value to be developed in the future for various industry including wastewater treatment industry.

Keywords: Lipase, copra waste, solid state fermentation, *Aspergillus Niger*

Introduction

Solid-state fermentation (SSF) is a valuable procedure transforming agro-industrial by-products into value added product of commercial interest. SSF is defined as any fermentation process performed on a non-soluble material that acts both as physical support as well as a source of nutrients in absence of free-flowing liquid (Costa *et al.,* 2018). The technique involves inoculation and growth of microbes on porous particulate solid substrate maintaining low moisture content. The water content and nutrients present in the substrate support the growth of microorganisms and the organisms secrete useful enzymes while growing on a solid substrate. Other than that, microbial growth is very depending on the temperature of the surrounding, thus the temperature has to be at specific range for metabolisms in the microorganisms to function normally and effectively (Pandey *et al,*. 2003).

The use of enzymes in the field of biotechnology and industry is increasing, therefore enzyme assessment needs to be done to be used in this field as a result of exploitation of agro-industrial waste as resources in SSF method. Enzyme properties are very specific for industrial application compared as compared to inorganic catalysts because enzymes have been widely used in various food and non-food industry processes. In addition, more than 70% of the chemical industry uses enzymes as catalysts. This is because the use of enzymes has several advantages, namely having activities that are selective, safe, easily controlled, can be degraded biologically, have high catalytic performance. In addition, the enzymatic reactions that occur do not produce

by-products and enzymes can be active at certain temperatures and pH, thus enzyme is very potential to replace chemical catalysts in the industrial field (Ananthi *et al.,* 2014). Lipase enzyme is one of the most extensively studied enzymes and the most in-demand enzymes for industry. This enzyme is needed by some industries, because it catalyses the production of fatty acids that are used as raw materials for the manufacture of detergents, manufacture of polymers and emulsifiers in the pharmaceutical industry. However, the commercial enzymes are expensive and not economical feasible for some small to moderate industry. Therefore, an alternative method to produce enzyme using waste as a substrate to reduce the capital cost in producing commercial enzyme is currently being explored (Praveen & Savitha, 2012).

Lipase can be produced using microorganisms by SSF techniques, especially moulds. Mould is a type of microbe that meets 80% of its substrate needs from carbon-chain macromolecules (Vakhlu & Kour, 2006). The use of fungi in the fermentation process allows for the overhaul of material components that are difficult to digest to become more available so that the nutritional value is also possible. The quality of fermented products depends on the type of microbes and the solid medium used. Lipase has been widely used for industrial application for flavour development and processing other foods, like meat, vegetables, fruit, baked foods, milk product and beer (Ananthi *et al.,* 2014). In dairy industry, lipases are used to modify the lengths of fatty acid chain that enhance the flavours of cheeses. Other than food processing industry, lipase is also applicable for wastewater treatment system. In 2006, Jeganathan *et al.* demonstrated that the O&G removal was found to be approximately 50% when lipase was used in pet food industrial wastewater treatment and the lipase was produced by *C. rugosa*.

Aspergillus niger is one of the applicable microorganism's producer of lipase from the mould group. In this study, a solid medium that can be used in growing *Aspergillus niger* is copra waste. Coconut which is processed into copra is used as raw material in the vegetable oil manufacturing industry. Copra production is produced traditionally in several stages, including stripping coconut shells, drying and smoking. In the processing process, it is often found that copra is mouldy and usually discarded as a solid waste. Worldwide coconut industry produced high volume of coconuts approximately 55,500,000,000 annually from 12,000,000 hectares areas worth USD 6 billion (Smith *et al.,* 2009). Despite its production and demand, the handling of coconut residues is among the poorest when more than 1 million tons of coconut residues are thrown in environment every year. Lipase can be produced by using the mouldy copra as a microbial source. Copra waste which contain high water content can be a good medium to support the growth of mould (Pandey, 2002).

Due to the environmental and economic crisis, in Malaysia, 5 million of fruits in year 2013 were generated. Large amount of copra waste produced can cause the increasing amount the waste. It also can affect the environmental and health issue when the mass generation of the waste is not being proper disposed. Less reported researched that copra waste was utilized as substrate in SSF study to produce lipase. Thus, this study implemented waste recovery concept in which waste is converted to beneficial product through SSF, which is lipase by using copra waste as a substrate. Finally, the potential of SSF using copra waste in producing lipase is evaluated based on the optimum operating condition and furthermore, the individual and interaction effects of related parameters (fermentation time, temperature, substrates concentration and inoculum size of *Aspergillus niger*) using full factorial design were evaluated. To determine the efficiency of lipase produced in removing selected pollutant in wastewater treatment, further study was conducted to determine the efficiency of FOG removal from food processing wastewater.

Materials and Methods

Production of Lipase Enzyme

The copra waste collected were dried in oven at 45 °C for 24 hours to remove the water content. The dried copra was grounded by blender to increase the total surface area of substrate and then sieved through a 200 standard mesh sieves to obtain the powder. 5g of copra powder was added to the flasks and mixed with the distilled water based on different moisture content (40 % and 60 %. The contents were then sterilized by autoclaved at 121°C for 20 minutes. After cooling, the flasks were inoculated with 2 ml of diluted *Aspergillus niger* which has different inoculum size and then incubated in incubator at different temperature (25^{o}C and 35^{o}C) for 120 hours. After producing the enzyme, the lipase activity was tested according to methodology explained by Vakhlu and Kour (2006).

Experimental Design Using Full Factorial Design

The full factorial design in the minitab was used to design the experiment. The full factorial design can list out all the possible combination of factors. In addition, the full factorial design can determine the number of samples that need to run. The 2k design was used. There were 3 factors in the study, and the samples were triplicate, thus 24 runs of experiments were performed.

Optimization of Lipase Enzyme

The optimization of lipase was carried out to determine the optimum growth condition for the maximum enzyme production in SSF and the lipase concentration was measured in U/ml. Therefore, three parameters were studied as listed in Table 1.

Table 1: Operating parameters for lipase enzyme optimization

Factors operating parameters	Range	
Temperature (°C)	25	35
Moisture content (%)	40	60
Inoculum size (petri dish)	1	9

The parameters were incubation temperature, moisture content and inoculum size. The incubation temperature that was studied were 25°C and 35°C whereas for moisture content were 40% and 60%. Furthermore, the study range for inoculum size were 1 petri dish and 9 petri dishes.

Food Processing Wastewater

The sample of food processing wastewater contained oily wastewater was collected from local fish cracker processing industry nearby Kuala Terengganu. The main characteristic of wastewater which is oil and grease (O&G) was analysed before and after the pre-treatment.

Pre-Treatment Process of Oily Wastewater Using Lipase Enzyme

The effects and efficiency of lipase on the removal of pollutants in food processing wastewater are determined based on O&G was carried out in batch test for 6 continuous days. O&G concentrations were determined based on Method 5520 Standard Method.

Results and Discussion

By using Minitab, a regression and calculation can be conducted statistically. Table 2 presents the coefficient of the model, standard deviation of the coefficient and probability for the full factorial designs.

Table 2: Coefficient of the model, standart deviation of the coefficient and probability for the full factorial designs

Term	Effect	Coef	SE Coef	T	P
Constant		135.63	0.5512	246.05	0.000
Temperature	-5.42	-2.71	0.5512	-4.91	0.000
Moisture content	-25.42	-12.71	0.5512	-23.06	0.000
Inoculum	4.58	2.29	0.5512	4.16	0.001
Temperature* Moisture Content	26.25	13.13	0.5512	23.81	0.000
Temperature*Inoculum	-2.08	-1.04	0.5512	-1.89	0.077
Moisture content*Inoculum	-2.08	-1.04	0.5512	-1.89	0.077
Temperature*Moisture Content*Inoculum	-3.75	-1.87	0.5512	-3.40	0.004

S = 2.70031 PRESS = 262.5
R-Sq = 98.64 % R-sq (pred) = 96.94 % R-Sq(adj) = 98.04%

The individual effect plot of temperature, moisture content and inoculum size are shown in figure 1. The individual effect plot was generated to represent the result of analysis. The individual effect shows the high and low level of each factor. When factor has positive effect, the response value increases as the factor changes from low to high level. Conversely, a reduction occurs for high level of the same factor if the effects are negative (Ponnusami *et al.,* 2007). The larger the gradient, the larger the change in response when changing from level -1 to +1.

As observed from figure 1, the two parameters which is temperature and moisture content have negative effect mean, while for the inoculum has positive effect from low level to high level. In conclusion, both temperature and moisture content individually show the highest performance at 25°C and 40% while for the inoculum size is 9 petri dishes. All affecting parameters depicted that all factors seem to affect the result because the line is possibly not horizontal.

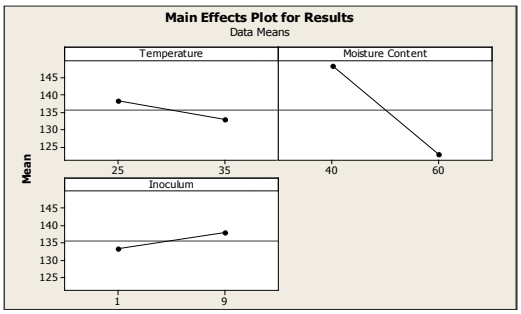

Figure 1: Individual effect plot for lipase enzyme production (U/ml)

An interactive effect is effective when change of response from low to high levels of factor is depend on the factor which the lines is not parallel (Mathialagan & Viraraghavan, 2005). Figure 2 shows the interaction effect of temperature, moisture content, and inoculum. All interactions were significant. Interactions between temperature and moisture content (AB) has strong interaction exists in these plots as the line of the graph cross each other. In the second plot and third plots, interaction between temperature*inoculum (AC) and moisture content*inoculum (BC) shows a weak interaction since both lines do not cross each other. These two factors give low effect. In conclusion interaction between temperature*moisture content was stronger than other interactions, while the interaction between moisture content*inoculum (BC) was the weakest.

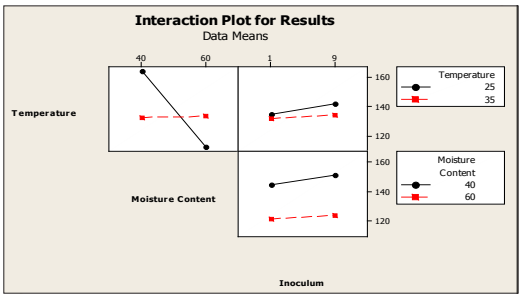

Figure 2: Interaction effects plot for lipase enzyme production (U/ml)

Figure 3 shows the 3D response surface plot interaction of moisture content and temperature. The contour plot depicted in Figure 4 used to

interpret the data from the response surface plot. In contour plot of result versus moisture content and temperature, the dark green region reflecting the highest reading which exceed 160 in condition moisture content is 40% and the temperature is 25°C while the dark blue region represented the lowest reading which is below 110 in condition 60% and temperature 25°C. The curve line in the contour plot shows that there was strong interaction exist between moisture content and temperature.

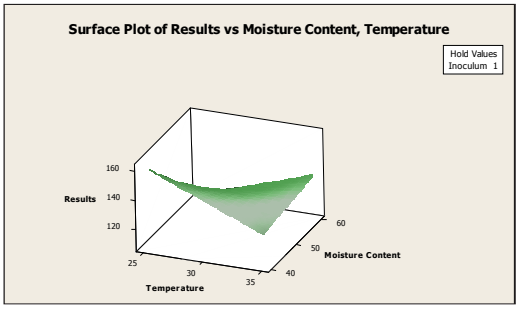

Figure 3: 3D Response surface plot of the moisture content and temperature

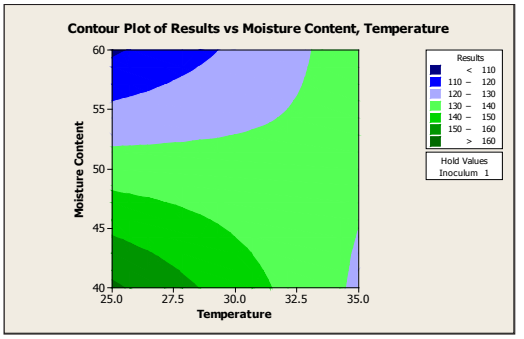

Figure 4: Contour plots of the moisture content and temperature

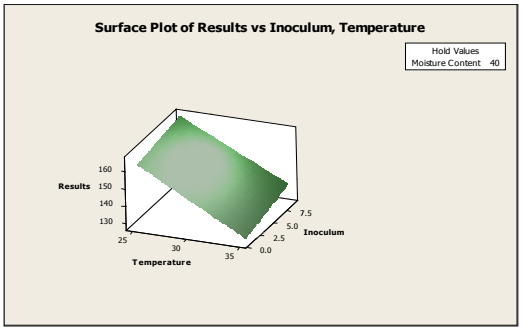

Figure 5: 3D Response surface plot of the inoculum and temperature

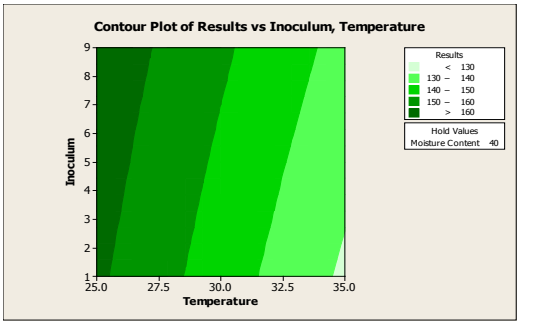

Figure 6: Contour plots of the inoculum and temperature on the lipase production (U/ml)

Figure 5 and 6 illustrates 3D response surface plot and contour plot with interaction between inoculum and temperature, respectively. To interpret the data, contour plot of result versus inoculum and temperature was fully analysed. Highest efficiency achieved in the greenish region which is exceed 150 and the lowest efficiency found in the blue region which is below than 130.

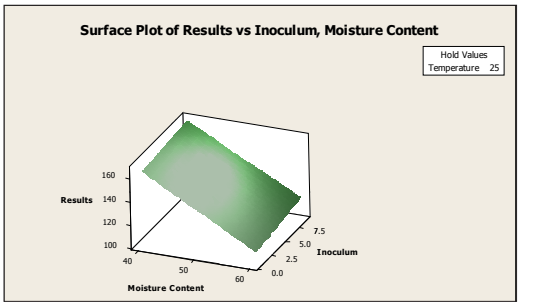

Figure 7: 3D Response surface plot of the inoculum and moisture content

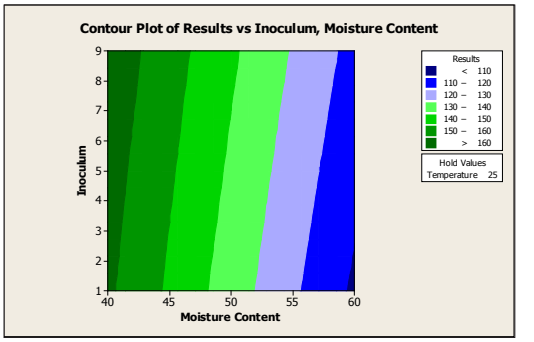

Figure 8: Contour plots of the inoculum and moisture content on lipase production (U/ml)

3D response surface of interaction between temperature and moisture content- has been plotted in Figure 7. Contour plot in Figure 8 were used to analyse results versus temperature and moisture content-. The greenish region indicates the highest efficiency which is exceed 140 in the condition high moisture content combined with the blue region reflecting the lowest efficiency which is below than 120.

Lipase being produced from the optimization processes was applied to the food processing wastewater treatment. Lipase is functioned to remove the FOG concentration that contained in the wastewater. The actual concentration of FOG was measured before and after the treatment. Figure 9 shows the trends of FOG concentration in wastewater for 6 consecutive days. As depicted, the concentration was reduced from 219.4925 mg/l to 169.467mg/l. Thus, the amount of FOG removal is 34%.

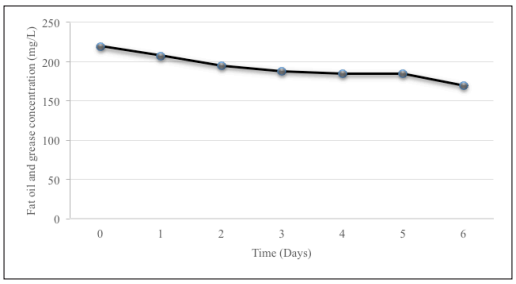

Figure 9: Concentration of fats, oil and grease (mg/L) in food processing wastewater treatment with lipase

Conclusion

The individual and interaction effect of selected parameter on temperature, moisture content and inoculum are significant towards lipase concentration. The highest lipase activity was recorded at 170 U/ml. The optimum condition for the lipase enzyme production in SSF from this study is 30 ºC the incubation temperature, 40 % of the moisture content of substrates and inoculum of the *Aspergillus niger* is 9 petri dishes. For the wastewater treatment, the concentration of the FOG in food processing wastewater shows the total reduction of 34%. In conclusion, lipase enzyme produced from SSF on copra waste substrate using *Aspergillus niger* is potential with SSF method and applicable to be used in wastewater treatment system for the removal of specific pollutant such as O&G.

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