Optimisation of biodiesel production of Black Soldier Fly larvae rearing on restaurant kitchen waste

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Abstract. The objective of the study was to optimise the biodiesel production of Black Soldier Fly Larvae (BSFL) rearing on restaurant kitchen waste (RKW). The BSFL was inoculated into the RKW and left for 20 days. After that, the BSFL were harvested, rinsed, inactivated and oven-dried. The extraction of larval crude oil from BSFL was conducted using Soxhlet method with several solvents, namely petroleum ether, acetone and ethanol. Petroleum ether and reaction time of 6 hours were the best extraction conditions to produce larval crude oil production of up to 56 %. Then it was underwent two-step transesterification process to produce biodiesel. There are four experimental variables which were optimised using the Response Surface Methodology (RSM) based on central composite design. A quadratic model was employed to predict the biodiesel yield, where the R² value was found to be 0.99 that reveal the satisfactory accuracy of the model to fit the experimental data. Based on optimisation studies, the optimum experimental conditions to obtain up to 96% of biodiesel from BSF larval oil were methanol:oil molar ratio of 9:1, catalyst concentration of 1 weight %, reaction time of 68 minutes and temperature of 60 °C.

1. Introduction
Energy resources are play important roles in the development of every country in the world. The evolution of energy growth is affected by economic and population growth, energy prices and fuel availability [1-3]. Unfortunately, the big challenge in the usage of fossil fuels is non-renewable resources that gradually depleting in reserves and related to the environmental degradation issue [3]. Biodiesel offers a promising alternative renewable fuel due to its advantageous of renewability, higher combustion efficiency, nontoxic, higher biodegradability and lower in sulphur and aromatic content [4,5]. Biodiesel is a monoalkyl ester of long chain fatty acid derived from renewable lipids such as vegetable oils, waste cooking oils, microorganisms and animal fats [6,7]. Nowadays, about 95% of global biodiesel production is made from vegetable oils. However, the large scale of biodiesel production from vegetable oils may increase the cost for raw material due to the fact that more land is required for plantation. Vegetable oil derived biodiesel may also cause imbalance to the food supply and market demand [7,8].

Thus the study on biodiesel production using insect larvae rearing on restaurant kitchen waste is believed to be an available biodiesel feedstock at the lower cost. Black soldier fly larvae are naturally feed in organic wastes, incorporating the nutrient into their bodies and reducing the amount of waste material in the rearing process [9,10]. Transesterification is the most well-known method for biodiesel production due to its simplicity and requires a lower cost. Transesterification process involves a reaction between triglyceride molecules with an alcohol in the presence of the catalyst to form esters and glycerol. A two-step transesterification is ideal for high Free Fatty Acid (FFA) oil in order to
eliminate soap formation during alkali-catalysed transesterification. There are several factors that may influence the rate of transesterification reaction such as methanol:oil (molar ratio), catalyst amount (weight %), reaction time (min) and reaction temperature (°C). RSM has been applied to determine the optimum condition for the operating variables. RSM also has the capability to investigate the effect of different independent parameters as well as minimising the experimental run with suitable proof for the result to be accepted statistically. Therefore, the aim of this study was to optimise the biodiesel production of BSFL rearing on restaurant kitchen waste using RSM.

2. Materials and methods

2.1. Cultivation of BSFL
Cultivation of BSFL was carried out under open barrel at temperature of 25-32 °C and humidity of 60-75%. After 20 days of cultivation period, BSFL were harvested by separating the BSFL from the restaurant kitchen waste, rinsed with deionised water and inactivated at 105 °C for 5 minutes followed by oven-dried at 60 °C for 24 hours.

2.2. Extraction study
Extraction of BSF larval oil was performed in a Soxhlet system consisted of 500 mL round bottom flasks, holder, siphon tube, condenser and hot plate. Figure 1 shows the (a) BSF larvae. About 30 g of dried BSFL was placed in holders with different solvent (250 mL petroleum ether, 250 mL ethanol and 250 mL acetone), separately. This solvent was subjected to Soxhlet treatment at different extraction time ranging from 1 to 6 hours. BSF larval oil was then obtained by removing the solvent using distillation process. The round bottom flask was placed in the oven at 103 °C and heated to constant weight followed by cooling in the desiccators for 30 minutes. The round bottom flask containing the boiling chips and BSF larval oil were weighed and the percentage of BSF larval oil produced was determined. The extracted BSF larval oil may contains impurities including phospholipids and solid impurities. After extraction, BSF larval oil was treated with 1% (v/v) of concentrated H₃PO₄ (85%, v/v) at 30 °C and 2-4% of softened water and mixed accordingly. Finally, the BSF larval oil was centrifuged to separate the pectin. High purity of BSF larval oil was collected after the refinement process.

Figure 1. (a) BSF larvae, (b) Dried BSF larvae, and (c) Soxhlet system.

2.3. Production of biodiesel
Biodiesel production was accomplished using a two-step transesterification process; acid-catalysed esterification and alkaline-catalysed transesterification due to high FFA content in the BSF larval oil. The acid-catalysed esterification step was a pretreatment used to lower the FFA content in the crude oil followed by alkaline-catalysed transesterification that converts the esterified oil to the biodiesel. The preliminary analyses of the acid-catalysed esterification and alkaline-catalysed transesterification were carried out according to a method outlined by Yang et al. [11] with some modifications. For
acid-catalysed esterification procedure, about 10 g of BSF larval oil was placed into a 250 mL of conical flask, heat to certain temperature and added with the mixture of methanol and sulfuric acid (H\textsubscript{2}SO\textsubscript{4}). The resulted solution was then stirred for certain reaction times followed by centrifuge at 400 rpm for 10 minutes. After acid-catalysed esterification, the upper layer was then transferred to a conical flask and dry in an oven at 105 °C for 10 minutes. The alkaline-catalysed transesterification was conducted by placing about 10 g of esterified oil into a conical flask, heat to certain temperature, added with the mixture of methanol and sodium hydroxide (NaOH ) and stirred for a certain reaction of times. The solution was then separated using the centrifuge at 400 rpm for 10 minutes. The upper layer (biodiesel) was measured and the percentage yield was recorded accordingly. Figure 1(b-c) presents the (b) BSF larval oil and (c) BSFL biodiesel.

2.4. Statistical analysis
In order to optimise the central composite design (CCD), a five-level four-factor central composite design was employed that generated thirty experimental runs. The aim of this analysis was to determine the optimum biodiesel yield (Y) as well as to investigate the effect of variables include methanol:oil molar ratio (A), catalyst amount (B), time (C) and temperature (D). The experimental condition range for RSM was determined based on the preliminary study. The data obtained from the experiments were analysed using RSM in order to fit the quadratic polynomial equation. Based on the response obtained, the statistical analysis such as analysis of variance (ANOVA) and regression coefficient (R\textsuperscript{2}) was accomplished.

3. Results and discussion

3.1. Extraction study
Solvent extraction is a well-established method for extraction of crude oil. The selection of an ideal solvent for extraction process is imperative in order to obtain high yield of BSF larval crude oil. Extraction study was carried out using three solvents, namely petroleum ether, acetone, and ethanol with different polarity properties. Figure 2 presents the percentage yield of crude oil extracted using these solvents. Following six hours of extraction, ethanol has resulted in the highest yield of BSF larval crude oil of up to 58%, followed by petroleum ether (56%) and acetone (50%). Although ethanol exhibited the best solvent to produce BSFL larval crude oil, it has been discussed by Wang et al. [12] that it has tendency to extract impurities such as phospholipids and solid precipitate. The presence of both phospholipids and solid precipitate will disfavour transesterification of crude oil to biodiesel. This scenario could be due to their ability to decrease the catalytic efficiency that may potentially reduce the biodiesel yield. As reported by López et al. [13] and Park et al. [14], non-polar solvent such as petroleum ether results in slightly lower yield than ethanol due to its ability to extract triglycerides only. Meanwhile, acetone also has potential in extracting lipids but from this experiment the application of acetone produced the lowest crude oil yield as compared to other solvents. This outcome consequently due to polarity properties of acetone itself that is more non-polar than ethanol and less non-polar than petroleum ether. Indeed, polar and non-polar lipids dissolve in acetone but not as high as ethanol and petroleum ether.

Reaction time also plays an important role in the extraction of crude oil. As shown in Figure 2, there was a marginal increase in crude oil yield when the extraction time was increased from 1 to 6 hours. For example, the percentage yield of crude oil extracted by ethanol after 4, 5 and 6 hours of extraction was determine as 42, 46 and 58%, respectively. A similar trend was also reported by Feng et al. [15]. The marginal increment trend was discussed due to washing and diffusion phases that involved in extraction process. Washing phase refers to washing of lipid on insect body surface by solvent. While, diffusion phase involves the mass transfer of solid-liquid from lipid to solvent.

Based on its ability to extract triglycerides only, petroleum ether was chosen as a solvent to extract BSFL crude oil in this study. Meanwhile, 6 hours was set as an optimum extraction time for extraction
process. It has been discussed by Tan et al. [16] that the increase in extraction time can be considered uneconomical and time-consuming procedure. Furthermore, an increase in extraction time will lead to loss of solvent during vaporisation.

Figure 2. Extraction of BSF larval crude oil.

3.2. Optimisation of transesterification
Table 1 presents the results of the design matrix with 30 experiments and experimental yields using CCD. It is apparent that the combination of each variable significantly influenced the biodiesel yield. The five replicates (center point) were used to indicate the experimental error and reproducibility of obtained data. Analysis of variance (ANOVA) for CCD model is presented in Table 2, which is useful in verification of the significance and fitness of the quadratic model. The model F-value of 103.68 and Prob>F of <0.0001 implied that the mode was significant at 95% confidence level. ANOVA results also identified that A, B, C, D, AB, AC, AD, BC, BD, CD, A², B², C², and D² were significant model terms. The lack of fit F-value of 1.63 and p-value of 0.3085 indicate that the model is satisfactory fitted to experimental data. The regression statistic goodness of fit (R²=0.9898) represents the reasonable agreement between the predicted yield and the actual yield. The relationship illustrated in the graph of predicted yield against experimental yield is shown in Figure 3 (a). Biodiesel yield including the experimental run and outlier t plot are shown in Figures 3 (b,c). Adequate precision (AP) is a measure of the signal to noise ratio by comparing the range of predicted values at the design points to the average prediction error. The AP value obtained from this study was determined as 35.362, which suggesting a desirable ratio. As discussed by Patel et al. [17] an AP value which greater than 4 indicates adequate model discrimination.

3.2.1. Effect of methanol:oil molar ratio. Stoichiometrically, one mole of triglyceride (TAG) requires 3 mol of alcohol in transesterification. However, due to reversible nature of the reaction, excess alcohol is usually used in the transesterification in order to shift the reaction to the product side [18]. Figures 4 (a,b,c) present the 3D plot for the interaction effect between A: methanol:oil, B: catalyst amount, C: time and D: temperature on biodiesel yield. The plot showed that high biodiesel yield (>90%) can be obtained between methanol:oil molar ratio (8:1 to 10:1). Generally, further increase of the methanol:oil molar ratio (11:1 to 12:1) leads to decrease of biodiesel yield. This might be due to excess methanol in the reaction mixture tends to increase the solubility of glycerol hence promoting the reverse reaction between glycerol and ester that reducing the biodiesel yield [19].
Table 1. Design matrix of experiments and experimental yields using CCD.

| Run | Methanol:oil (molar ratio) | Catalyst amount (%) | Time (min) | Temperature (ºC) | Biodiesel yield (%) | Run | Methanol:oil (molar ratio) | Catalyst amount (%) | Time (min) | Temperature (ºC) | Biodiesel yield (%) |
|-----|---------------------------|---------------------|------------|------------------|---------------------|-----|---------------------------|---------------------|------------|------------------|---------------------|
| 1   | 10:1                      | 1.0                 | 60         | 35               | 38                  | 16  | 8:1                       | 0.5                 | 80         | 45               | 77                  |
| 2   | 8:1                       | 1.5                 | 80         | 45               | 66                  | 17  | 8:1                       | 1.5                 | 80         | 65               | 88                  |
| 3   | 8:1                       | 0.5                 | 80         | 65               | 63                  | 18  | 12:1                      | 0.5                 | 80         | 65               | 53                  |
| 4   | 10:1                      | 1.0                 | 60         | 55               | 90                  | 19  | 10:1                      | 1.0                 | 60         | 55               | 95                  |
| 5   | 8:1                       | 1.5                 | 40         | 45               | 24                  | 20  | 8:1                       | 0.5                 | 40         | 65               | 84                  |
| 6   | 12:1                      | 1.5                 | 80         | 65               | 85                  | 21  | 8:1                       | 1.5                 | 40         | 65               | 60                  |
| 7   | 10:1                      | 1.0                 | 60         | 55               | 90                  | 22  | 10:1                      | 1.0                 | 100        | 55               | 80                  |
| 8   | 10:1                      | 1.0                 | 20         | 55               | 60                  | 23  | 12:1                      | 0.5                 | 80         | 45               | 50                  |
| 9   | 10:1                      | 1.0                 | 60         | 55               | 94                  | 24  | 12:1                      | 1.5                 | 40         | 45               | 28                  |
| 10  | 6:1                       | 1.0                 | 60         | 55               | 66                  | 25  | 8:1                       | 0.5                 | 40         | 45               | 73                  |
| 11  | 10:1                      | 1.0                 | 60         | 55               | 94                  | 26  | 14:1                      | 1.0                 | 60         | 55               | 53                  |
| 12  | 12:1                      | 1.5                 | 40         | 65               | 81                  | 27  | 12:1                      | 0.5                 | 40         | 65               | 83                  |
| 13  | 12:1                      | 0.5                 | 40         | 45               | 56                  | 28  | 10:1                      | 0.2                 | 60         | 55               | 80                  |
| 14  | 12:1                      | 1.5                 | 80         | 45               | 52                  | 29  | 10:1                      | 2.0                 | 60         | 55               | 53                  |
| 15  | 10:1                      | 1.0                 | 60         | 55               | 95                  | 30  | 10:1                      | 1.0                 | 60         | 75               | 83                  |

Table 2. Analysis of variance (ANOVA) for CDD model.

| Source            | Sum of squares | Degree of freedom | Mean of square | F-value | Prob>F |
|-------------------|----------------|-------------------|----------------|---------|--------|
| Model             | 11515.79       | 14                | 822.56         | 103.68  | < 0.0001 significant |
| A-Methanol:oil    | 222.04         | 1                 | 222.04         | 27.99   | < 0.0001 |
| B-Catalyst amount | 305.78         | 1                 | 305.78         | 38.54   | < 0.0001 |
| C-Time            | 301.04         | 1                 | 301.04         | 37.94   | < 0.0001 |
| D-Temperature     | 2838.37        | 1                 | 2838.37        | 357.75  | < 0.0001 |
| AB                | 248.06         | 1                 | 248.06         | 31.27   | < 0.0001 |
| AC                | 232.56         | 1                 | 232.56         | 29.31   | < 0.0001 |
| AD                | 232.56         | 1                 | 232.56         | 29.31   | < 0.0001 |
| BC                | 1425.06        | 1                 | 1425.06        | 179.62  | < 0.0001 |
| BD                | 855.56         | 1                 | 855.56         | 107.84  | < 0.0001 |
| CD                | 430.56         | 1                 | 430.56         | 54.27   | < 0.0001 |
| A²                | 1867.32        | 1                 | 1867.32        | 235.36  | < 0.0001 |
| B²                | 1210.87        | 1                 | 1210.87        | 152.62  | < 0.0001 |
| C²                | 864.30         | 1                 | 864.30         | 108.94  | < 0.0001 |
| D²                | 1755.35        | 1                 | 1755.35        | 221.25  | < 0.0001 |
| Residual          | 119.01         | 15                | 7.93           |         |        |
| Lack of fit       | 91.01          | 10                | 9.10           | 1.63    | 0.3085 Not significant |
| Pure error        | 28             | 5                 | 5.60           |         |        |
| Cor total         | 11634.80       | 29                |                |         |        |

* R² : 0.9898  Adjusted R² : 0.9802  Adequate precision : 35.362
3.2.2. Effect of catalyst amount. As discussed by Issariyakul and Dalai [18], the application of catalyst will enhance the transesterification process by accelerating the breaking of triglycerides (TAG) bond. This reaction comprise of three steps: (1) attack of alkoxide ion to the carbonyl carbon of the TAG to form a tetrahedral intermediate, (2) the tetrahedral intermediate reacts with alcohols to regenerate the alkoxide ion, and (3) rearrangement of the tetrahedral intermediate to form alkyl ester and diglycerides (DGA) that continues to change to monoglycerides (MAG). Study on interaction of catalyst amount between A: methanol:oil, C: time and D: temperature on biodiesel yield are represented in Figures 5 (a,d,e). The 3D plots showed that high biodiesel yield (>90%) can be obtained between catalyst amount (0.5 to 1.0 weight %). However, an increase in catalyst amount up to 1.5 weight % has resulted in a gradual decrease trend on biodiesel yield. Rajan et al. [20] were reported that high catalyst amount may reduce the biodiesel yield due to soap formation that may have caused difficulties for glycerol separation from biodiesel.

3.2.3. Effect of reaction time. Reaction has been regarded as one of the main part to be considered in transesterification. The results for interaction study between reaction time with A: methanol:oil, B: catalyst amount and D: temperature on biodiesel yield are depicted in Figures 4 (b,d,f). The 3D plot (d) showed that high biodiesel yield (>90%) can be obtained between reaction time (40 to 60 min). Prolong the reaction time for 80 min with increasing of catalyst amount up to 1.5 weight % would decrease the biodiesel yield. Kafuku and Mbarawa [21] reported that decrease in biodiesel yield at longer reaction time due to the acceleration of ester hydrolysis and saponification with the alkaline catalyst. On the other hand, interaction between reaction time with methanol:oil and temperature showed slightly different results. The high biodiesel yield (>90%) may favour between 60 to 80 min. This could be related to insufficient reaction time to reach the equilibrium as shown in 3D plots (b,f).
3.2.4. **Effect of reaction temperature.** Figures 4 (c,e,f) illustrate the interaction between reaction temperature and A: methanol:oil, B: catalyst amount, and C: time on biodiesel yield. The 3D plot showed that high biodiesel yield (>90%) can be obtained between reaction time (55 to 65 °C). The reduction of biodiesel yield was observed at higher temperature (75 °C) that might be due to vaporisation of methanol resulting in a two-phase interface in the reaction mixture. Moreover, the possibility of side reaction of alkyl ester hydrolysis also may occur and produce acids at the high temperature that able to decrease the biodiesel yield [22].

![3D plots](image)

**Figure 4.** 3D plots represent the interaction between each variable on biodiesel yield: (a) methanol:oil and catalyst amount, (b) methanol:oil and time, (c) methanol:oil and temperature, (d) catalyst amount and time, (e) catalyst amount and temperature and (f) time and temperature.
3.3 Optimisation of biodiesel production

Optimisation study was carried out to determine the optimum variables for the maximum biodiesel yield. In this study, biodiesel yield was set to a maximum value, while other variables were set in a range between high and low levels. The optimum biodiesel yield of 97% was achieved by transesterification of BSF larval oil with methanol:oil molar ratio of 10:1, catalyst amount of 1.1 weight %, reaction time of 61 min and reaction temperature of 62 °C. RSM with CCD was considered suitable for prediction of optimised biodiesel yield and transesterification with small percentage error (1.11%).

4. Conclusion

RSM with CCD was successfully applied in optimisation of biodiesel production from BSFL using several variables. It was found that biodiesel yield up to 97% was obtained at optimum variables of 10:1 (methanol:oil molar ratio), 1.1 weight % catalyst amount within 61 minutes of reaction time and 62 °C of reaction temperature. Besides, the BSF larval crude oil was ideally extracted using petroleum ether as a solvent and 6 hours of reaction time.

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