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Optimisation of Lipid Extraction from Primary Sludge by Soxhlet Extraction

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Domestic sewage sludge is a potential raw material to produce energy in the form of renewable biofuels (biodiesel) due to the high amount of lipid content in the sludge. Soxhlet extraction is commonly used in extracting lipid from primary sewage sludge. Different solvents and extraction parameters in Soxhlet extraction may affect the lipid yield. Hexane, methanol and toluene were evaluated in extracting lipids from primary sewage sludge. Methanol was identified as the most suitable solvent to be used in lipid extraction due to its high polarity. Extraction parameters such as temperature, extraction time, and sludge-to-solvent ratio were investigated in order to optimise the lipid extraction process from primary sewage sludge. The extracted lipid yield was observed to be optimum at 40.21 wt% when the temperature and extraction time were at 86 °C and 7.45 h. A sludge-to-solvent ratio of 2:1 was determined to be the optimum value in extracting lipid yield. The optimisation process was conducted using response surface methodology (RSM) and was analysed using ANOVA with R^2 of 0.8146 for lipid concentration. The analysis of free fatty acid content in the lipid extracted from primary sewage sludge revealed that the composition of free fatty acid was dominated by monounsaturated oleic acid.

1. Introduction

Sewage sludge is a solid, semisolid, or liquid muddy looking residue that results after plain sewage is treated at a wastewater treatment plant. It is mainly produced from domestic and industrial activities. Malaysia has found to produce approximately 3×10^6 m^3/y of sewage sludge in wastewater treatment plants in these recent years and the amount is keep increasing (Abu Hasan et al., 2012). As a result, many new sludge treatment and disposal facilities are needed to manage the large volume. Abu Hasan et al. (2012) estimated that the sewage sludge produced in Malaysia will increase to 7 Mt in year 2020. The estimation for the total cost in managing sewage sludge is about 1 B MYR (Mohd Safuan et al., 2014).

Sewage sludge is a good source of micro/macronutrients for plants besides its richness in organic matter and beneficial to the soil, crops, and livestock productivity. However, the use of sewage as a fertiliser is restricted in many countries due to bad odour and the presence of heavy metals and toxic substances. Recently, domestic sewage sludge has been used and proven to be a good raw material to produce energy in the form of renewable biofuels such as biodiesel and is considered to be economically and eco-friendly process; although the process itself is quite complicated (Girisha et al., 2014). Sewage sludge contains significant concentrations of lipids derived from the direct adsorption of lipids onto the sludge (Kargbo, 2010). The amount of lipid-rich wastewater increases every year due to urbanisation (Chipasa and Mędrzycka, 2006).

Lipids are one of the most important components of natural foods that constitute one of the major types of organic matter found in municipal wastewater which may find their way into surface waters. Therefore, an alternative to sewage management and disposal challenge is to utilise it as a source of lipid feedstock for biodiesel production (Jardé et al., 2005). However, the compositions of lipids in sewage sludge are different according to the stage of wastewater treatment that been passed through. The presence of microorganisms

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during wastewater treatment will also affect the composition of lipids due to the phospholipids component of the cell membrane of the microorganisms. As a raw material for lipid extraction, sludge can be collected at primary, secondary, or tertiary treatment system. Most of the studies done by other researchers have extracted lipid from sewage sludge by using primary and secondary sludge (Pastore et al., 2013).

Due to the great chemical complexity and diversity of the sludge produced, the lipid extraction becomes difficult (Bharathiraja et al., 2014). Many investigations have been done by other researchers to solve this problem using different methods to extract the lipid from sewage sludge. Olkiewicza et al. (2014) studied that the solvent extraction using methanol can produce 14.16 % of lipid from primary sewage sludge. However, study on the optimisation of operating parameters for lipid extraction from primary sludge by Soxhlet extraction has not been done yet. Therefore this study was performed to determine the optimum parameters of temperature, time, and sludge-to-solvent ratio in Soxhlet extraction using response surface methodology (RSM).

2. Materials and Method

2.1 Wastewater sample and samples drying

Sludge samples were collected from one of the primary waste water treatment plants in Johor, Malaysia. Sludge samples (15 L) were taken manually and kept in a closed sealed plastic container. The moisture content of the sludge sample was 96 %. The samples were immediately transported back to the laboratory for immediate use. Sewage sludge was dried first before being used for the extraction process. Twenty grams of wet sample was mixed with 0.3 mL fuming hydrochloric acid. After the acidification process, 25 g of magnesium sulphate monohydrated was added to the mixture. The mixture was stirred in order to obtain a smooth paste and it was left until solidified (15 - 30 min). Then, the dried sample was ground using a pestle and mortar until it was homogenous before filled into cellulose thimble for extraction process.

2.2 Extraction of lipids

An empty thimble and an empty round bottom flask were weighed. After adding up the dried sludge (20 g) into the thimble, it was weighed again and the reading was recorded. Then, 200 mL of hexane was added into 250 mL round bottom flask. The heating process was monitored to allow 80 cycles of the extraction in approximately 6 h. The hexane was removed from the flask by rotary evaporator after the lipid had been extracted. Then, the lipids were stored in a desiccator overnight and weighed the next day. The yield of extracted material was determined gravimetrically and expressed as g of extractable lipids per g of dry sludge. The step was repeated with different solvents, which were methanol and toluene.

2.3 Optimisation of lipid extraction process by response surface methodology

The optimisation experiments were conducted for three factors with two numerical factors and one categorical factor with the levels of each factor are shown in Table 1. The factors were temperature, time, and sludge-to-solvent ratio. The response was extracted lipid yield with 22 set of experiments as shown in Table 2. Analysis of variance (ANOVA) was used for the analysis of the experimental data to determine the interactions between the process variables and response obtained as well as model validation. The quality of fitness in the polynomial model was expressed by the correlation coefficient (R^2) and its statistical significance was verified by an F-test. The model terms in this study were evaluated based on the P-value (probability) corresponding to a 95 % confidence level. The optimisation process was done with the aid of Design Expert Software for mathematical model and statistical analysis (Design Expert, 2005).

Table 1: Level of factors for the optimisation process

Factor	Level of value (Numerical Factor)		
	-1	0	+1
Temperature	70	80	90
Time (h)	4	6	8
	Level of value (Categorical factor)		
	Level	Level 1	Level 2
Sludge : solvent ratio	2	1:2	2:1

Table 2: Experimental data for the optimisation process

Run	Temperature (°C)	Time (h)	Solvent : solvent ratio	Run	Temperature (°C)	Time (h)	Solvent : solvent ratio
1	80	6	1:2	12	70	4	2:1
2	90	4	2:1	13	90	8	2:1
3	70	8	1:2	14	80	6	1:2
4	80	4	1:2	15	80	4	2:1
5	80	6	2:1	16	70	4	1:2
6	70	8	2:1	17	80	6	1:2
7	80	8	1:2	18	70	6	2:1
8	80	8	2:1	19	70	6	1:2
9	90	6	2:1	20	90	4	1:2
10	90	6	1:2	21	80	6	2:1
11	90	8	1:2	22	80	6	2:1

2.4 Total primary sludge lipid content

The lipid content was calculated as the total crude lipid yield for each experimental set. The extracted samples was calculated as percentage based on Eq(1).

$$\text{Extraction (\%)} = (w_2 - w_1) / (m_2 - m_1) \times 100 \quad (1)$$

Where m_1 represents the mass (g) of thimble, m_2 is the total mass (g) of thimble and sample (g), w_1 is the mass (g) of round bottom flask, and w_2 is the total mass of round bottom and lipid after extraction (g)

2.5 Free fatty acid analysis

Gas chromatography with flame ionisation detector (GC-FID) was used to determine the free fatty acid contents in primary sewage sludge oil. Primary sewage sludge oil (5 mL) was allowed to dry and reconstituted in 200 µL of ethanol. In this chromatographic separation, helium gas was used as the carrier with 0.9 mL/min flow rate. The sample volume used for injection was 1 µL.

3. Results and discussion

3.1 Lipid yield of different solvent extraction

Sewage sludge contains various solute molecules with more than one functional group so it was difficult to predict the solubility of the solutes in particular solvents. The polarity concept of the solvent was used to solve the problem of solubility. In this study, the lipid extracted from primary sewage sludge was successfully performed only using methanol. Both n-hexane and toluene were not able to extract the lipids from the primary sewage sludge at described conditions. Meanwhile, 10.5 % of lipid was successfully extracted by using methanol. The non-polar characteristic of hexane may have come out this observation. However, high polarity of methanol breaks the weak phospholipid bond of the sludge sample by the reaction of methanol on the cell membrane of the microorganisms in the sewage sludge. It results in the separation of lipid from the phospholipid bond. Normally, high polarity solvent that has high value of polarity index is able to extract a high amount of lipid oil from primary sewage sludge. Therefore, methanol was able to extract a higher fraction of the more polar free fatty acids, whereas n-hexane appeared to be a better extraction agent for the non-polar glycerides (Melero et al., 2015).

3.2 Optimisation of lipid extraction using RSM

The optimisation process was done using response surface methodology (RSM). The results for 22 runs are shown in Table 3. Based on the sequential model sum of square, the model for lipid yield was selected based on the highest order linear equation. The models were coded X1 for lipid yield percentage and the independent variables in the models were temperatures (A), reaction time (B), and sludge-to-solvent ratio (C). The final empirical models used to generate coded factors for each variable is shown in Eq(2). In addition to the ANOVA statistical analysis, the quality of the model was also evaluated based on the coefficient of determination. The ANOVA results for the quadratic model for lipid yield percentage are shown in Table 5.

Table 3: Response value for different experimental conditions

Run	A = Temperature (°C)	B = Time (h)	C = Solvent : solvent ratio	Lipid yield (%)
1	80	6	1:2	33.50
2	90	4	2:1	31.00
3	70	8	1:2	32.01
4	80	4	1:2	26.90
5	80	6	2:1	37.80
6	70	8	2:1	34.50
7	80	8	1:2	36.50
8	80	8	2:1	38.80
9	90	6	2:1	37.60
10	90	6	1:2	34.60
11	90	8	1:2	38.00
12	70	4	2:1	27.30
13	90	8	2:1	40.00
14	80	6	1:2	34.90
15	80	4	2:1	28.50
16	70	4	1:2	27.90
17	80	6	1:2	31.90
18	70	6	2:1	33.50
19	70	6	1:2	30.10
20	90	4	1:2	28.10
21	80	6	2:1	37.80
22	80	6	2:1	38.10

$$\text{Lipid Yield (\%) } R1 = 33.57 + 2.0A + 4.18B + 1.39C + 0.95AB + 0.22AC + 0.24BC - 0.98A^2 - 2.26B^2 \quad (2)$$

In this study the value of the determinations coefficients (R^2= 0.9428) indicates that only 5.72 % of the total variations are not explained by the model. The models obtained from the design fitted well with the experimental data. The value of the adjusted determinations coefficients (Adj R^2 of 0.9076) was also high, which indicates a high significance of the model. Based on the ANOVA result in Table 4, the parameter gives a significant effect on the lipid yield percentage. The model F-value of 26.78 implies the model is significant due to Prob > F, which is less than 0.01 % chance of the "Model F-value" to occur due to noise.

Table 4: Analysis of variance for lipid yield percentage

Source	Sum of square	Mean square	F value	P-value Prob>F	
Model	347.49	43.44	26.78	< 0.0001	Significant
A – Temperature	47.96	47.96	29.57	0.0001	
B – Time	209.25	209.25	129.03	< 0.0001	
C – Ratio	42.26	42.26	26.06	0.0002	
AB	7.20	7.20	4.44	0.0551	
AC	0.57	0.57	0.35	0.5642	
BC	0.70	0.70	0.43	0.5238	
A^2	4.90	4.90	3.02	0.1057	
B^2	25.85	25.85	15.94	0.0015	
Residual	21.08	1.62			
Lack of fit	16.52	1.84	1.61	0.3417	Not significant
Pure error	4.57	1.14			
Cor Total	368.58				
Std Dev	1.27				
R^2					0.9428
Adj R^2					0.9076

3.3 Extraction efficiencies

To assess the interactive relationships between independent variables and the responses in the model, a 3D surface response and contour plots were utilised using Design Expert software. As shown in Figure 1, the optimum value observed for lipid yield is 40.21 % using a process condition of sludge-to-solvent ratio of 2:1 at 86 °C for 7.45 h.

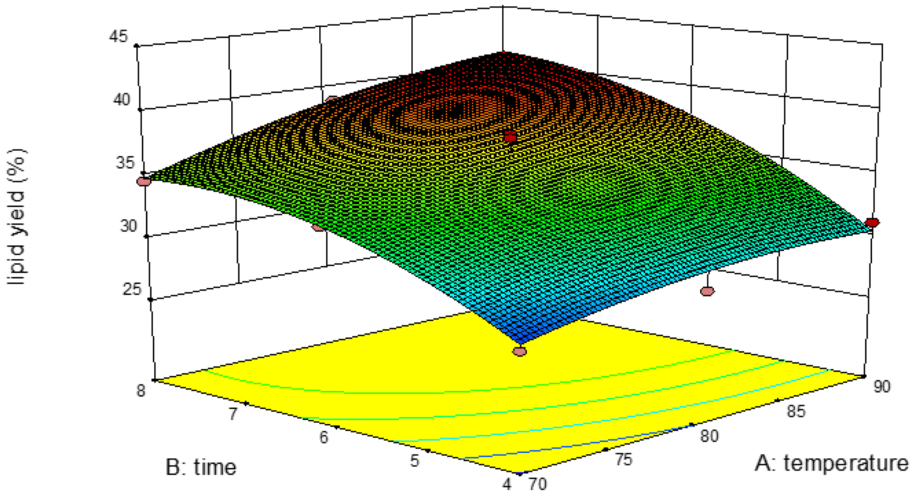

Figure 1: Response surface for lipid yield percentage. Effect of reaction time and temperature at fixed ratio (2:1)

3.4 Effects of temperature, extraction time and ratio sludge to solvent on extracted lipid yield

By referring to the data show in Table 5, the experimental data was in the range with previous research. Thus primary sewage sludge was proved as suitable raw material in lipid extraction. It is expected that a higher lipid yield could be obtained due to the significant effect of temperature and reaction time that had been determined in the optimisation process. Besides, the longer the extraction period, the higher degree of adsorption of lipid from the sewage sludge could be because highly non-polar lipid molecule in the sewage sludge need a longer time to be separated (Olkiewicza et al., 2015). However, the disadvantage of longer extraction period was the contamination with non-saponifiable matter which was not convertible into biodiesel (Olkiewicza et al., 2015).

Table 5: Lipid yield percentage by other researchers

Type of sludge	Method	Lipid yield (wt %)	References
Dried primary sludge	Soxhlet extraction	37.30	Bozhagian (2014)
Dried primary sludge	Soxhlet extraction	24.80	Pokoo-Aikins et al. (2010)
Dried primary sludge	Liquid-liquid extraction	26.60	Olkiewicza et al. (2014)
Dried primary sludge	Soxhlet extraction	27.20	Olkiewicza et al. (2015)
Raw primary sludge	Ionic liquid extraction	26.90	Olkiewicza et al. (2015)
Primary sludge	Solvent extraction	13.60	Melero et al. (2015)
Primary sludge	Soxhlet extraction	40.21	This study

3.5 Analysis of the free fatty acid content

Siddiquee and Rohani (2011) stated that the most dominant free fatty acid contents in the lipid oil from primary sewage sludge was palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1). In this study, the dominant fatty acid was oleic acid with the highest concentration obtained at 77.1 ppm. The concentration of palmitic acid was too small which was not significant to be identified. The monounsaturated oleic acid gives strong oxidation stability of biodiesel. The saturated or monounsaturated fatty acids, such as palmitic acid, stearic acid, and oleic acid, promote oxidation stability. The polyunsaturated fatty acids, such as linoleic acid and linolenic acid, tend to give a poor oxidation stability. Most of the biodiesel from the polyunsaturated fatty acid are difficult to achieve the minimum limit of six hours for oxidation stability (Olkiewicza et al., 2012). However, the melting points of biodiesel product depend on the chain length and the degree of unsaturation, therefore the polyunsaturated fatty acids will have a favourable cold flow properties (Melero et al., 2015,). Most of the researchers used oleic acid as a raw material to produce biodiesel (Kusmiyati et al., 2010). According to Tran et al. (2013), oleic acid is known to be a main component of biodiesel, accounting for more than 50 % of the weight of total fatty acids. The nature of monounsaturated oleic acid gives an advantage in the oxidation stability and cold flow properties (Patel and Narkhede, 2012).

4. Conclusion

Methanol was preferable as solvent in extracting lipid from primary sewage sludge. The optimisation process for lipid extraction process using methanol was affected by temperature, extraction time, and sludge-to-solvent ratio. The extraction process was successfully performed and yielded an increased amount of lipid up to 40 % from 10.5 % before the optimisation process. The analysis of free fatty acid content in the lipid extracted from primary sewage sludge revealed that the composition of free fatty acid was dominated by monounsaturated oleic acid.

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