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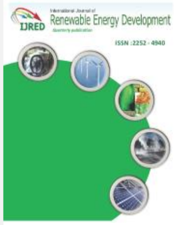

Optimization of Lipid Extraction from Municipal Scum Sludge for Biodiesel Production Using Statistical Approach

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ABSTRACT. Design of Experiment (DoE) as a statistical method was applied for optimizing lipid extraction conditions from scum sludge. Four different extraction variables were optimized namely methanol to hexane ratio (%), solvent to sludge ratio (ml/g), temperature (°C), and extraction time (h). Process optimization was conducted through three main steps: 1) 2^k factorial screening design; 2) Steepest ascent method; and 3) Box-Behnken design and response surface method. Based on 2^k factorial screening design, methanol to hexane ratio, solvent to sludge ratio and temperature were identified as highly significant variables affecting lipid extraction from scum sludge. Based on screening results, the steepest ascent method was used followed by Box-Behnken design and Response Surface Method (RSM) were then applied for optimization. The maximum extracted lipid of 29.4% (wt lipid/wt dry sludge- %) was achieved at 40% methanol to hexane ratio (%), 40 solvent to sludge ratio (ml/g), 90°C and 6 hours extraction time. The results revealed that lipid extraction increases with reducing the methanol to hexane ratio, increasing solvent to sludge ratio and increasing temperature The results demonstrated the potentiality of scum sludge for biodiesel production from scum sludge compared with the amount of lipid extracted from primary and secondary sludge reported by other studies.

Keywords: Box-Behnken design, Design of Experiment (DoE), lipid extraction, Scum sludge, response surface method (RSM).

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1. Introduction

Currently, most of the energy we use originally comes from fossil fuels (non-renewable energy sources) which projected to be depleted in the near future (Shafiee & Topal, 2009; Siddiquee & Rohani, 2011a). Despite our dependence on fossil fuels as energy sources resulted in a number of serious environmental problems, the energy demand for fossil fuels has been increasing during the last few years, and expected to further increase in the future (Revellame, Hernandez, French, Holmes, & Alley, 2010). All these factors have led to global interests in developing biodiesel as an alternative fuel. Biodiesel is regarded as promising green fuel to replace an appreciable amount of traditional petroleum-based diesel fuel. It is renewable, biodegradable, less toxic, and safe for storage and handling, and characterized by energy density equivalent to currently used petroleum-derived diesel and can be used directly without any modifications in the currently used engine or new refueling stations.

Chemically, biodiesel fuels are fatty acid methyl or ethyl esters (FAME) that is produced via esterification and transesterification of various lipid sources in the presence of a base, acid, enzyme or solid catalyst. Biodiesel can be generated from different natural resources such as vegetable oils or animal fats. Biodiesel has been used as fuel in diesel engines and heating systems for over 25 years (Atabani et al., 2012; Banković-Ilić, Stojković, Stamenković, Veljkovic, & Hung, 2014; Bharathiraja, Chakravarthy et al., 2014; Dufreche et al., 2007; Ingole & Kakde, 2012;

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Leiva-Candia et al., 2014, 2015). However, the competitive potentiality of biodiesel is limited by the high price of conventional lipid feedstocks they produced from, which constitutes between 70% and 85% of the overall current biodiesel production cost (Sheik, Muller, & Wilmes, 2014). Furthermore, the cultivation of edible vegetable oils for biofuels raises the concerns of food shortage, which competes with fuel production, therefore, at present, biodiesels cannot compete economically in the market (Atabani et al., 2012). Therefore, a low-cost and non-edible feedstock is required to reduce the production costs and facilitate competitiveness with petroleum diesel.

Municipal sludge lipids are gaining more attention nowadays as a promising source for lipid which can lower biodiesel cost production and make it more profitable for industries. As municipal sewage sludge is a waste, formed during the treatment of wastewater, it is a possible alternative source of lipids for the production of biodiesel, consequently lowering the wastewater treatment plant (WWTP) operation costs (Bharathiraja, Yogendran, Ranjith Kumar, Chakravarthy, & Palani, 2014; Kargbo, 2010; Kargbo, 2010; Olkiewicz, 2015; Revellame et al., 2011; Sheik et al., 2014). Research has shown that the lipids contained in sewage sludge are a potential feedstock for biodiesel mainly and comprised of fatty acids predominantly in the range of C10 to C18, which are excellent for the production of biodiesel (Kargbo, 2010; Mondala, Liang, Toghiani, Hernandez, & French, 2009a).

Lipid extraction is the first step for biodiesel production from wastewater treatment plant sludge. At present, several methods are available for lipid extraction from biological materials. Most of these methods use organic solvents, usually in mixtures (Boocock, Konar, Leung, & Ly, 1992; Dufreche et al., 2007; Pokoo-Aikins et al., 2010; Siddiquee & Rohani, 2011a, 2011b). Extraction of lipids from sludge can be influenced by many variables such as the type of sludge, type and amount of solvent, extraction time, temperature, stirring rate, type of microorganisms present in the sludge, etc. Although several researchers have demonstrated the lipid extraction from wastewater sludge, the effects of different parameters and their optimization have not been investigated.

Several authors have investigated biodiesel production from primary sludge (Kargbo, 2010; Mondala et al., 2009a; Mondala, Liang, Toghiani, Hernandez, & French, 2009b; Olkiewicz et al., 2014, 2015; Pastore et al., 2013; Qi et al., 2015; Siddiquee & Rohani, 2011a), as well as secondary sludge (Bharathiraja et al., 2014; Kumar, Ghosh, Khosla, & Thakur, 2016; Mondala et al., 2009b; Revellame et al., 2011; Siddiquee & Rohani, 2011a). Only a few studies payed attention to scum sludge as a potential source for biodiesel production (Bi et al., 2015; di Bitonto, Lopez, Mascolo, Mininni, & Pastore, 2016; Wang, Feng, Bai, Zhao, & Xia, 2016). Scum sludge is the floatable material skimmed from the surface of grit champer, primary and secondary settling tanksin wastewater treatment plants. It consist mainly of fat, oil and grease that are washed from the plumping system. Around 1.9 gallon/person/year scum sludge is generated according to Wiltsee (1998). Scum sludge causes several operational troubles such as system cloging, floatation of sludge, deterioration of aerobic and anaerobic processes and deteriorating the performance of wastewater treatment plant (Chipasa & Mędrzycka, 2006).

In the present study, lipid extraction from scum sludge was investigated for optimization using four variable which believed to play a significant role in the extraction of lipid using organic solvents. solvents used in lipid extraction was a mixture of methanol (was used a polar solvent) and hexane (was used as a non-polar solvent). Lipid extraction process was optimized using Design of Experiment (DoE) approach. The Four factors investigated in the current study were: 1) methanol to hexane ratio (%) (X1); 2) solvent to sludge ratio (X2); 3) temperature (X3); 4) and extraction time (X4). Screening of main effects was evaluated to out-screen exctraction variables with insignificant effects from the optimization process. To our knowledge the results obtained in the current study were the first in optimizing lipid extraction from scum sludge.

2. Materials and Methods

2.1. Sludge Sample collection and preparation

The scum sludge used in this study was collected from screening chamber of municipal wastewater treatment located in Madinah, KSA, in Alkhaleel area north of the city. The treatment plant is a conventional activated sludge system (without tertiary treatment) and has a design capacity of 129.000 m^3/d. In 2001, the WWTP was upgraded to a total capacity of 240.000 m^3/d and include extended aeration activated sludge tank followed by sand filtration system (Fig. 1).

The raw sludge samples were collected from the scum screening chamber in plastic bottles and kept on ice during transportation to the laboratory. Scum samples were filtered and concentrated by gravitational settling at 5°C for 12 hours. After settling the supernatant was discarded and the settled sludge was subjected to two rounds of centrifugation at 3000 rpm for 10 min for further dewatering. The thickened sludge samples were then frozen at -20°C and freeze-dried for 5 days. The freeze-dried samples were crushed using mortar and pestle, homogenized and then stored in freezer till further use.

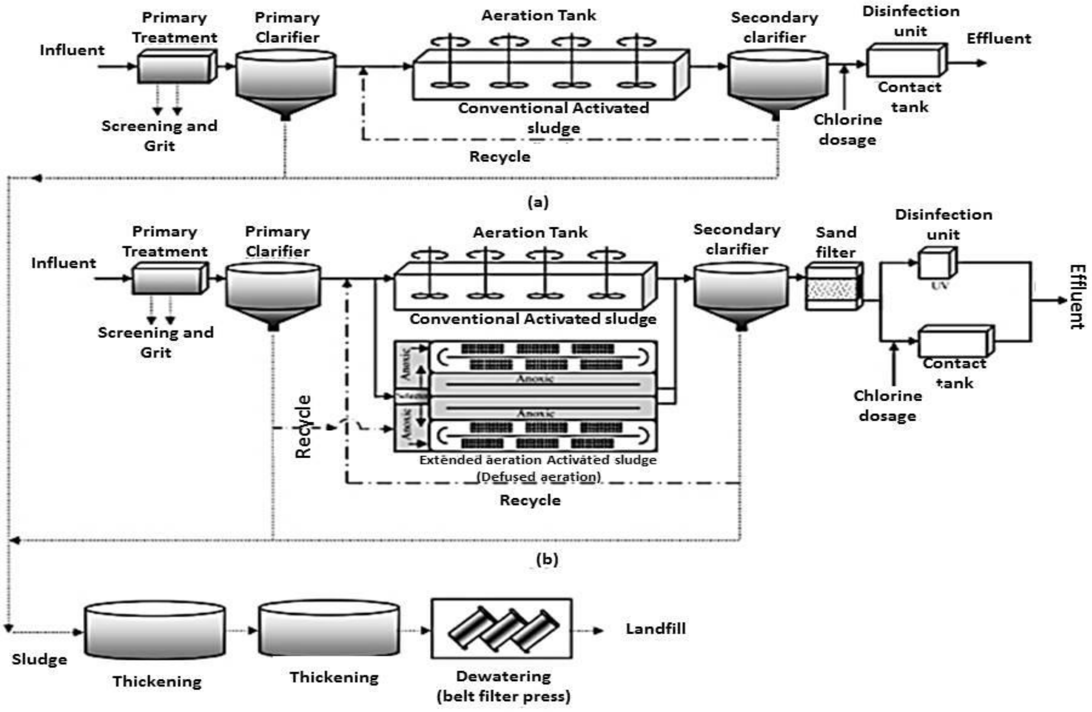

Fig. 1. A schematic layout for Madinah WWTP (Al Saleem, 2007)

2.2. Lipid Extraction

The layout of experimental approach adopted in the current study is shown in Figure 2. Lipid extraction was conducted using different extraction variables as listed in Table 1, namely, methanol to hexane ratio (%), solvents to sludge ratio (ml solvents/g dried sludge), temperature (^{o}C) and extraction time (h). Lipid extraction from scum sludge was carried out according to the method described by Wang et al. (2016). The mixture predefined amount sludge power and solvents (based on the experimental design level) was placed in a condenser-attached 500 ml Erlenmeyer flask for sequential extraction. After extraction, the resulting mixture was filtered using Buchner funnel, Whatman filter paper No. 1 and water aspirator to remove the remaining solvents. The filtrates were further concentrated using a rotatory evaporator at 40oC and dried to a constant mass in a vacuum desiccator. The resulting lipid was weighed, and the yield of extracted lipid was then determined and expressed as percentage of grams extractable lipid per gram dry sludge (Dufreche et al., 2007). All solvents (Methanol and n-Hexane) were HPLC-grade and purchased from Fisher Scientific (Atlanta, USA). The yield of extracted lipid was calculated according to the following formula:

$$Y = \frac{Residual\ weight\ (mg)}{Sludge\ solid\ weight\ (mg)} \times 100 \qquad (1)$$

2.3. The Design of Experiment (DoE) for lipid extraction from scum sludge

The Design of Experiment (DoE) approach allows investigating the influence of different factors on a given process, through conducting minimal number of experiments (Imandi, Bandaru, Somalanka, Bandaru, & Garapati, 2008). DoE as a statistical approach has been widely applied and successfully implemented for different bioprocess optimization purposes (Annadurai, Mathalai Balan, & Murugesan, 1999; Francis et al., 2003; Imandi et al., 2008; Kumar et al., 2016; Long et al., 2010; Siddiquee & Rohani, 2011a; Varrone et al., 2012). Process optimization using DoE usually involves four main steps: 1) independent variables selection and their variation ranges and defining dependent (response) variable(s); 2) data screening for determining the main effect independent variables on response variable; 4) carrying out the statistically designed experiments in a randomized order and estimating the coefficients and the mathematical model; 5) checking, verifying and optimizing the resulting model. In the current study different statistical methods were used to investigate and optimize four extraction variables to increase the extracted lipid content (yield, Y). The statistical methods used included 2k factorial screening design to determine the main effects and Response Surface Method (RSM) for modeling the process and optimization. All statistical analysis was performed using the JMP@ software (Version 13.1.0, SAS Institute, Cary, NC, USA).

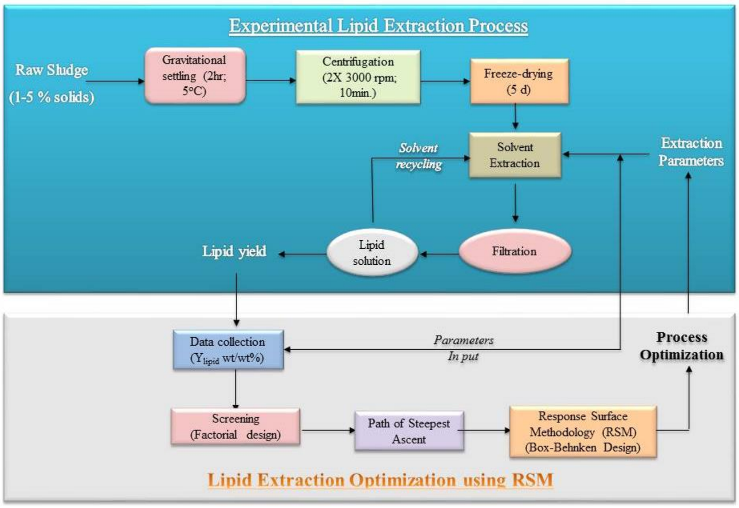

Fig. 2. Schematic representation of experimental lipid extraction process and extraction optimization steps using DoE.

2.4.2^k factorial screening design

Screening the main effects of variables is the first step in the optimization process, in which the effect magnitude of every independent variable was estimated. 2^k factorial design as a screening method is well established statistical technique for screening the main effects of the independent variables based on first order model (Siddiquee & Rohani, 2011a):

$$Y = \beta_0 + \sum \beta_i X_i + \varepsilon \qquad (1)$$

Where Y is the response variable (Y_{lipids} wt/wt%) , β_0 is the model intercept, β_i is the linear coefficient, X_i is the magnitude of the independent variable, and ε is the error factor.

According to 2^k factorial screening design, four extraction variables were screened using full factorial analysis with 16 runs with two levels design: low (-1) and high (+1). Table 2 and 3 represents the screening design matrix illustrating the levels and significance of factors respectively. The main effect of each variable was estimated based on the average difference between the high and low levels measurements. All experimental measurements for the screening experiments were performed in triplicates and averaged. Analysis of variance (ANOVA) was conducted to evaluate variables with a significant effect on lipid yield from the extraction process (response variable, Y_{lipid}).

2.5. The path of steepest ascent

The path of steepest ascent method was adopted to determine the direction of the increases in response variable (Y_{lipid}). Determining the direction of increase helps to direct the experimental region of response towards optimum by changing the range of selected variables. The direction of response increase was determined based on the results obtained from the screening step, which identified the significant variables. A step-wise steepest ascent was performed started from the variable levels that produced the maximum lipid yield in the screening results, and ended when a near optimal/plateau point was reached. The results from the steepest ascent step were further used for further process optimization using response surface method (RSM).

2.6. Box–Behnken design for lipids extraction optimization

For optimizing the lipid extraction from scum sludge, Box–Behnken design and Response surface methodology (RSM) were applied (Kumar et al., 2016; Varrone et al., 2012). Box-Behnken design (BBD) was performed to optimize the most significant variables identified by factorial screening design step with three center points. Table 4 shows the experimental design matrix used for Box–Behnken design. The Response Surface Method (RSM) was thus applied to visualize the experimental region. Predicting the optimal conditions can be estimated using the following second-order polynomial equation:

$$Y_{lipid} = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j + \varepsilon \qquad (2)$$

where Y_{lipid} is the predicted response variable (amount of extracted lipid from scum sludge), β_0 , β_i, β_{ii} *and* β_{ij} are the regression coefficients of intercepts, linear quadratic and interaction terms respectively. While X_i and X_j are the independent variables and ε is the error term. Measurements for optimization experiments were performed in triplicates and averaged except for the center points. The quality of the fit of the polynomial model equation was determined using regression coefficient (R^2) and Adjusted R^2. The significance the regression coefficients were checked using F-test.

3. Results and Discussions

3.1 Screening of variables for lipid extraction from scum sludge using factorial design

The 2^k screening designs experiments and the resulting Y_{lipid} of 16 runs are shown in Table 2.The effects of initial methanol to hexane ratio, solvent to sludge ratio, temperature and extraction time on lipid yield Y_{lipid} (wt lipid /wt sludge %) are represented in Table 2. The analysis of variance (ANOVA) indicated that the resulting model fit for Y_{lipid} was highly significant (p= <0.0001), besides the model lack of fit value was > 0.05 (p=0.5371) and R^2= 0.98 confirming that the model fits the experimental data and explains 98% of the data variability. According to Table 2, methanol to hexane ratio (X1), solvents to sludge ratio (X2) and temperature (X3) showed a significant effect on Y_{lipid} (p<0.05). In particular, methanol to hexane ratio and solvents ratio showed the highest effect. Where extraction time, showed small or no effect on Y_{lipid} within the examined range (p>0.05). Therefore, extraction time variable (X4) was excluded from the steepest ascent test. All examined variable showed a positive effect on Y_{lipid} as shown by the sign of β coefficient, except for methanol to hexane ratio (X1) which showed a negative correlation as indicated in Table 3. The highest Y_{lipid} recorded was at 40:40:80:6 for X1, X2, X3 and X4 respectively. Indicating that at lower methanol to hexane ratios higher amounts of lipids can be extracted. Similar results was recorded by Wang et al. (2016), who reported 33% lipid yield from scum sludge using similar ratios.

Table 1.
The experimental variables, their low, high and center point levels for lipid extraction from scum sludge

Variables	Low (-1)	High (+1)	Center point (0)
X1: Methanol to Hexane (%)	40	80	60
X2: Solvent to Sludge ratio (ml/g)	10	40	25
X3: Temperature (° C)	30	80	55
X4: Extraction Time (h)	1	6	3.5

Table 2.
2^k factorial screening design matrix and the response variable (Y_{lipid}) from scum sludge.

Run order	X1	X2	X3	X4	Y_{lipid} (wt/wt %)
1	-	+	-	-	27.4
2	+	-	-	-	19.8
3	-	-	+	-	27.4
4	+	-	+	+	23.4
5	-	-	-	-	24.4
6	-	+	+	+	29.21
7	+	+	+	+	25.7
8	+	-	+	-	23.3
9	-	+	-	+	27.9
10	+	+	-	-	21.8
11	+	+	-	+	22.1
12	-	+	+	-	29.1
13	+	-	-	+	20.1
14	+	+	+	-	24.7
15	-	-	-	+	24.8
16	-	-	+	+	27.6

Table 3
Levels of variables examined and screening statistical analysis results.

Code	Variable	Low Level (-1)	High level (+1)	F-ratio	β coeff.	p-value
X1	Methanol to Hexane ratio (%)	40	80	883.819	-2.3069	0.0001*
X2	Solvent to sludge ratio	10	40	189.922	1.0693	0.0003*
X3	Temperature (°C)	30	80	317.141	1.3819	0.0003*
X4	Extraction time (h)	1	6	5.494	0.1819	0.0885

**indicates variables with significant effects on Y_{lipid} (p-value<0.05)*

According to Wang et al. (2016), the solvent with highest percentage of hexane (20:60:20) generated the largest lipid amount from scum sludge due to the dominance of neutral lipid in scum sludge which are easily extracted by hexane (non-polar solvent).

3.2 Process optimization using steepest ascent and response surface method (RSM)

According to the regression analysis of the screening design, the path of the steepest ascent was applied to determine the appropriate variables range to maximize the amount of lipid extracted. The steepest ascent experiments were designed based on the maximum Y_{lipid} recorded during the screening step (run 9). Accordingly, levels of significant variables were increased towards maximum lipid extraction region. A stepwise increase in the levels of solvents to sludge ratio and the temperature was carried out (Table 5; runs 5-8). Similarly, a stepwise decrease in the levels of methanol to hexane ratio was performed to estimate the optimal ratio for the highest Y_{lipid} (Table 4; runs from 1 -4). From Table 5, the maximum amount of extracted lipid was obtained at methanol to hexane ratio of 40%, solvent to sludge ratio of 40 ml/g and temperature at 90°C. It can be clearly seen that the levels of the three variables initially screened were close to optimum.

Box-Behnken design and Response surface method (RSM) were applied to optimize and model lipid extraction yield (Y_{lipid}) using three independent significant variables, namely, methanol to hexane ratio, solvent to sludge ratio and temperature. Process optimization was carried out using different levels of variables and Box-Behnken design as shown in Table 6 and 4 respectively, where a multiple regression analysis was applied to the experimental data in order to model Y_{lipid} . The second order polynomial equation (Eq. 3) was used to model the correlation between the significant variables identified during the screening step, and the response variable (Y_{lipid}). The quadratic equation model for the significant variables is shown by Eq.4.

Table 4
Box-Behnken design matrix for optimization step and the response variable (Y_{lipid}) from scum sludge.

Run order	X1	X2	X3	Y_{lipid} (wt/wt %)
1	+	0	+	29.3
2	0	-	-	28.2
3	+	0	-	28.4
4	-	0	-	27.4
5	0	+	+	29.4
6	-	0	+	28.8
7	0	-	+	28.4
8	0	0	0	29.35
9	0	+	-	28.7
10	0	0	0	29.27
11	-	-	0	27.8
12	+	+	0	29.2
13	+	-	0	28.6
14	0	0	0	29.31
15	-	+	0	28.4
16	0	0	0	29.25

Table 5
Steepest ascent experiments for maximizing lipid extraction from Scum sludge.

Runs	X1	X2	X3	Y_{lipid} *(wt/wt%)*
1	40	40	80	29.25
2	35	40	80	29.27
3	30	40	80	29.33
4	25	40	80	29.31
5	40	40	85	29.34
6	40	40	90	29.37
7	40	50	80	29.28
8	40	55	80	29.25

Table 6: Levels of variables used in Box-Behnken design.

Code	Variable	Low Level (-1)	High level (+1)	Centre point	F-ratio	p-value
X1	Methanol to Hexane ratio (%)	30	50	50	27.363	0.0016*
X2	Solvent to sludge ratio	25	55	40	27.752	0.0019*
X3	Temperature (°C)	73	90	73	29.157	0.0217*

**indicates variables with significant effects on Y_{lipid} (p-value<0.05)*

$$Y_{lipid} = -18.312686 + 0.556603X_1 + 0.048376X_2 + 0.794245X_3 - 0.004975X_1^2 - 0.001322\,X_2^2 - 0.004464\,X_3^2 - 0.001471\,X_1\,X_3 + 0.00098\,X_2\,X_3 \quad (4)$$

Where Y_{lipid} is the corresponding extracted lipid yield (wt/wt %). X_1, X_2, X_3 and X_4 represents the values of methanol to hexane ratio (%), solvent to sludge ratio (ml/g), and temperature (°C) respectively. The analysis of variance ANOVA showed that the model fit was highly significant (*p-value*=0.0025) and the lack of fit was not significant (p-value=0.5356) which shows the model represents the experimental data.

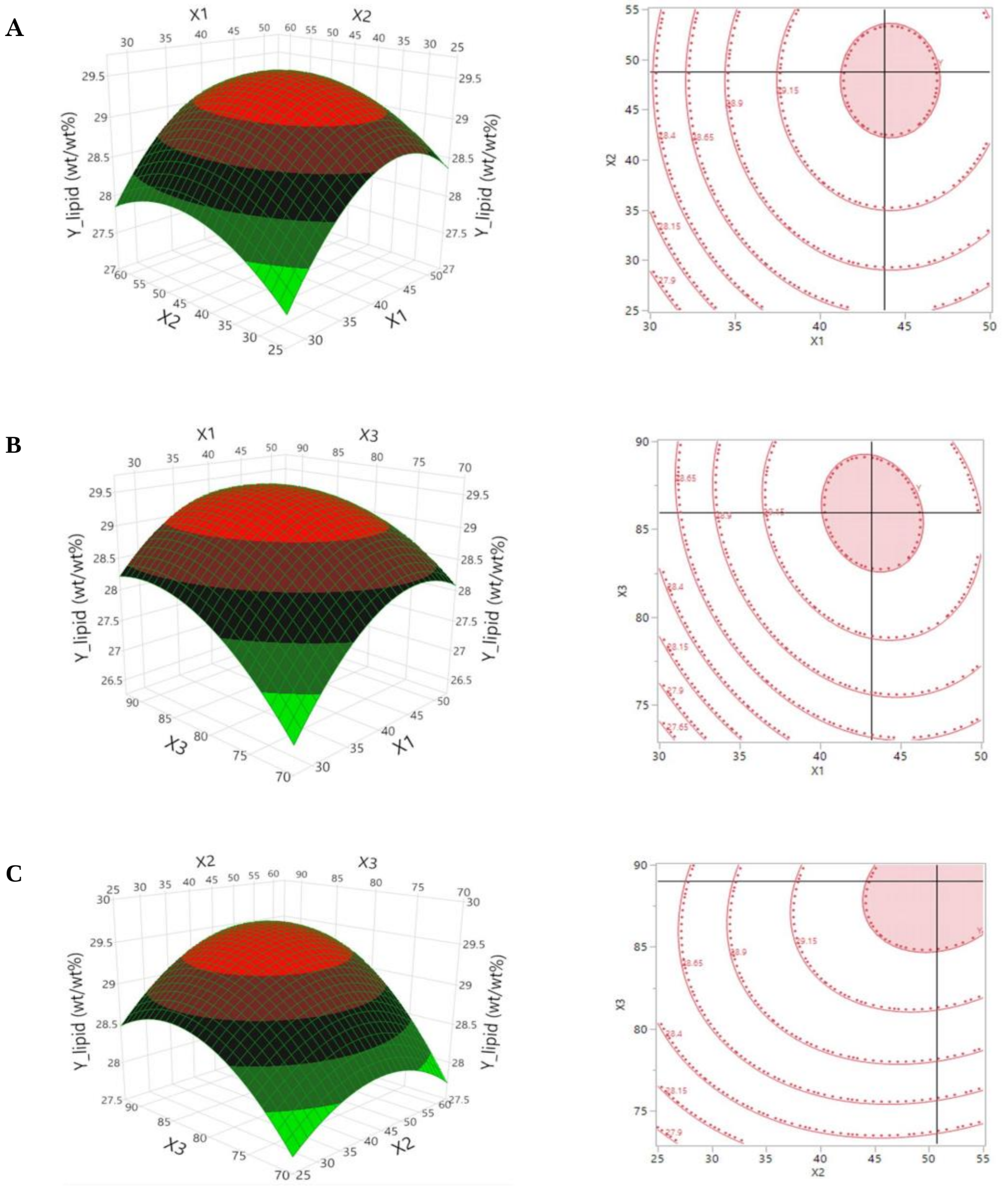

Fig. 3 Three-dimension surface plot and contour plot (A-C), showing levels of variables for maximizing lipid extraction yield from scum sludge.

The linear effect of methanol to hexane ratio (X1), temperature (X2) and solvent to sludge ratio (X3) were highly significant with *p*-values=0.0016, 0.0019 and 0.0217 respectively. Also, the quadratic effect of methanol to hexane ratio (X1) was as well highly significant (*p*-value=0.002) indicating the significant effect of this factor. While the linear effect of time (X4) was insignificant (*p*-value = 0.9591). The model coefficient (R^2) was found to be 0.952, explaining 95.2% of the variability of the response variable (Y_{lipid}) and with the Adj(R^2) = 0.88. The three-dimensional response surfaces which represents the effects of different variables on Y_{lipid} based on equation (4) is shown in Figure 3. Response surface shows the interactions between methanol to hexane ratio and solvent to sludge ratio, methanol to hexane ratio and temperature and solvent to sludge ratio and temperature (Fig. 3 A-C).

The response surface indicated that maximum yield of lipid from scum sludge could be achieved by decreasing methanol to hexane ratio, increasing solvents to sludge ratio and temperature. A clear peak was observed in the response surface for variables combinations indicating optimal conditions were achieved. The maximum amount of lipid extracted was 29.4% of dried scum sludge which is comparable to the results obtained from Wang et al. (2016) who extract 33.3% lipids from dry scum sludge using similar extraction conditions compared with a maximum yield of 27 and 17% for primary and secondary sludge respectively reported by the same study. According to the quadratic model prediction equation (eq.4), the maximum predicted extractable lipid was 29.58% (wt/wt%) at 42% methanol : hexane ratio, 50 solvent : sludge ratio and temperature of 87oC for 6 hours extraction time. This would indicate the potentiality of scum sludge for biodiesel production compared with the results obtained by other studies for lipid extraction from primary and secondary sludge (Mondala et al., 2009a; M. Olkiewicz et al., 2014, 2015; Revellame et al., 2010; Siddiquee & Rohani, 2011a).

According to a report of EPA's office of solid waste, in United States, about 1 to 3 billion gallon of grease and oil is yearly produced in 30 metropolitan areas, in which 60% enter the sewer system. Scum sludge, as mentioned before, has a negative impact on wastewater treatment process and the conventional treatment and disposal of scum sludge (either through co-digestion or landfilling) is not efficient and costly. Therefore, and from the results obtained from the current study, using of scum sludge as a feedstock for biodiesel production is the best currently available option.

4.Conclusion

The results presented in the current study indicated the potentiality of scum sludge as a feedstock for biodiesel production. The highest lipids extracted (29.4%) was achieved using 40:40:90:6 independent variables conditions (methanol to hexane ratio, solvent to sludge ratio, temperature and extraction time respectively). In addition, The results indicated that the amount of lipid extracted in the current study was higher in comparison with previous records from primary and secondary sludge. The results have showed that reducing methanol ot hexane ratio results in higher lipid yield due to the neutral nature of lipids dominating scum sludge. Also, the study has demonstrated the usefulness of applying Response Surface Method (RSM) approach for optimizing lipid-extraction process variables and was applied successfully in the current study. To our knowledge, the current results are among the best results reported so far for optimizing lipid extraction conditions from scum sludge.

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