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Optimization of decoloring conditions of crude fatty acids recovered from crude glycerol by acid-activated clay using response surface method

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Abstract−Crude glycerol, a by-product of the biodiesel production process, contains a high amount of fatty acids that cannot be used directly without removing the strong dark color. This study aims to remove the color impurities in crude fatty acids, prepared by acid-precipitation and hexane extraction, using acid-activated clay as an adsorbent. The effects of bleaching temperature, contact time, the amount of acid-activated clay, and concentration of color impurities were investigated. No significant effects of bleaching temperature or contact time were observed. In an optimization study using the central composite design, complete decolorization was achieved at an optimized condition, in which 3.5 g of clay was added to 10 mL of crude fatty acid solution with an optical density at 373 nm of 20. Notably, more than 80% of the peroxides was concurrently removed. However, a 37% loss in fatty acids was observed during decolorization. The clay was repeatedly used without loss of adsorption capability if it was calcined at 600 °C after use.

Keywords: Biodiesel, Crude Glycerol, Fatty Acid, Decolorization, Response Surface Design

INTRODUCTION

Crude glycerol is the principal by-product during the biodiesel production process [1]. Generally, 10 kg of glycerol is generated for every 100 kg of biodiesel produced [2]. As the demand and production of biodiesel are growing extensively, a worldwide surplus of glycerol has resulted and, thus, the price of crude glycerol has plummeted. Although refined glycerol can be obtained by purifying crude glycerol and then utilized in the cosmetic, paint, automotive, food, pharmaceutical, and pulp industries [1], it is not a complete solution because crude fatty acids are also generated. Intensive studies on bioconversion of crude glycerol to value-added products by means of fermentation have found that crude fatty acids are toxic to microorganisms and, thus, partially purified (fatty acid-free) crude glycerol is a better fermentation substrate than that of crude glycerol [3,4]. Therefore, we cannot avoid the generation of crude fatty acids as waste when utilizing crude glycerol. We have found a high amount of fatty acids salts (≥17%, w/w) in crude glycerol. The presence of these fatty acids may be due to neutralization of the free fatty acids present as impurities in oils by alkaline catalysts and concurrent saponification of oils during the biodiesel production process.

No industrial applications of these crude fatty acids have been proposed. Environmental problems would be created if these separated crude fatty acids were disposed. We found that palmitic acid (C16:0), oleic acid (C18:1), and linoleic acid (C18:2) are predominant in crude fatty acids generated from this process as mentioned in text. Fortunately, these long-chain saturated and unsaturated fatty acids can be used directly or as precursors for raw materials in cosmetics, pharmaceuticals, and fine chemicals [5-7]. However, the separated crude fatty acids have a strong dark brown color. If these crude fatty acids are intended for cosmetic applications, it is essential to remove the color impurities [8] but no processes are available for this purpose.

Adsorption processes are conventionally used to decolorize vegetable oils, in which the oil is contacted with adsorbents *in vacuo* in the presence of heat, followed by filtration [8,9]. Various kinds of adsorbents have been tested for refining vegetable oils. Although decoloring performance of activated carbon and silica-based adsorbents was better than other adsorbents, it was found that acid-activated bentonites (calcium-montomorillonite) are the most effective in removing color from the refined oils [8,10]. Therefore, this acid-activated clay was applied to decolorize the crude fatty acids in this study. Operating factors critically influence the performance of the decolorization process, so an evaluation and optimization of these factors is required. However, there is little information on optimization to obtain the highest decoloring performance. Furthermore, the excessive use of activated clay can cause environmental problems due to increased land-fill disposal [11].

This study aims to achieve complete decolorization of crude fatty acids (≥99%) using acid-activated clay with minimum generation of waste clay. In preliminary experiments, the effects of temperature and contact time were investigated. Central composite design (CCD) was applied as a statistical approach to determine the optimal amount of clay and initial color intensity of the crude fatty acid solution. It was also examined whether clay could remove toxic peroxides from a fatty acid solution. Finally, a simple regeneration method is proposed for the repeated use of clay.

MATERIALS AND METHODS

1. Materials

Crude glycerol was obtained from Eco Solution (Seoul, Korea).

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Table 1. Physical properties of acid-activated clay[a]

Free moisture (%)	6-10
pH (5%, sol.)	3-5
Acidity (KOH, mg/g)	<2.5
Particle size (%) (200 mesh pass)	>90
Specific surface area (m^2/g)	200-250
Bulk density (g/L)	550-650

[a] Data were obtained from a supplying company

Acid-activated clay (DC-A3) was purchased from Donghae Chemicals (Seoul, Korea). This clay is powder-type particle (200 mesh pass) with a high surface area. It was produced by acid treatment of calcium-montmorillonite and has been popularly used for refining edible oils (animal and vegetable oils). The physical properties of this clay are summarized in Table 1. Hexane (95% n-hexane) and HCl (purity, 35%) were purchased from J. T. Baker (Phillipsburg, NJ, USA) and Samchun Chemicals (Pyeongtaek, Korea), respectively.

2. Preparation of Crude Fatty Acids from Crude Glycerol

Fatty acids were prepared from crude glycerol following the methods described by Wanasundara and Shahidi [12] with some modification; 200 mL of crude glycerol was mixed with 200 mL of distilled water. The pH of the mixture was adjusted to 1.0 by adding HCl to convert the soup (fatty acid salt) to free fatty acids, which were precipitated from solution. Hexane (400 mL) was added to the acidified solution, the mixture was transferred to a separatory funnel, and the liberated fatty acids were extracted into the hexane. A hexane layer containing fatty acids was collected, and the hexane was removed in a rotary evaporator (EYELA N-1100, Rikakikai Co., LTD., Tokyo, Japan) at 55 °C for 40 min under vacuum. A sticky, dark-brown colored crude fatty acid solution (38 mL) was obtained, and this concentrated solution was suitably diluted with hexane for decolorization.

3. Decolorization of the Crude Fatty Acid Solution

All experiments were carried out in 250 mL Erlenmeyer flasks containing the crude fatty acid solution and acid-activated clay by maintaining the mixture at 100 rpm in a shaking incubator (Changshin Scientific Co. Ltd., Seoul, Korea). To examine the effect of temperature and contact time, 2 g of clay and 10 mL of suitably diluted crude fatty acid solution (optical density value at 375 nm [OD_{375}]=38) were mixed and maintained at two different temperatures (30 and 70 °C). After 30 or 120 min, 1 mL of sample was taken, the clay was separated by centrifugation (9,800 ×g for 10 min), and the supernatant color intensity was measured. CCD was applied to determine the optimal amount of clay and initial color intensity of the fatty acid solution. Fourteen experiments were performed in duplicate according to this design. Regression analysis of the data was conducted using SAS version 9.1 software (SAS Institute, Cary, NC, USA), and the optimal values of these factors were obtained by solving a second-order polynomial equation and analyzing the response surface contour plots.

The clay and fatty acids were separated by passing the slurry through a 0.2 mm nylon membrane filter (47 mm, Whatman International Ltd., Maidstone, England) under vacuum and the recovered clay was dried at 80 °C for 12 h. This spent clay was added to an unbleached crude fatty acid solution with or without calcination at 600 °C for 6 h, and the decolorization cycles were repeated to determine reusability.

4. Analyses

The color intensity of the crude fatty acid solution was measured with a UV-visible spectrophotometer (Hewlett-Packard, Palo Alto, CA, USA). The concentrated crude fatty acid stock solution was diluted in hexane, and the OD_{375} was determined using hexane as a reference after measuring the full spectrum of the sample between 200-500 nm. Decolorization efficiency (%) was calculated using the following formula:

$$\text{Decolorization efficiency (\%)}=(A_{unbleached}-A_{bleached})\times 100/A_{unbleached}$$

where $A_{unbleached}$ and $A_{bleached}$ are the OD_{375} values of the samples before and after decolorization with clay, respectively.

Fatty acid composition was determined for the crude fatty acid solution with or without decolorization. After esterification to fatty acid methyl esters (FAME) using methanolic HCl [13], the FAME samples were analyzed by gas chromatography (M600D, Yong-Lin, Seoul, Korea) equipped with a flame-ionization detector and a fused silica capillary column (100 m×0.25 mm I.D.×0.2 μm film thickness, SP™-2560, Supelco, Belafonte, PA, USA). Nitrogen was used as the carrier gas at a flow rate of 2.7 mL/min. The oven temperature was initially 140 °C for 5 min and was then raised to 240 °C at a rate of 4 °C/min and held for 10 min. The injector and detector temperatures were kept at 240 and 260 °C, respectively. Sample volume was 1 μL with a split ratio of 10 : 1. The fatty acids were identified by comparing the retention times with those of standard fatty acids (Supelco 37 Component FAME Mix). Changes in the area of the sample before and after decolorization were calculated to estimate the loss of fatty acids during the bleaching step. Concentrations of hydrogen peroxide (H_2O_2) and *tert*-butyl hydroperoxide (t-BuOOH) were determined by a colorimetric reaction using a commercial kit supplied by Sigma (St. Louis, MO, USA). Aqueous solutions of hydrogen peroxide (30%) and t-BuOOH (70%) (Sigma) were used for preparing the calibration curve, respectively.

RESULTS AND DISCUSSION

1. Selection of a Suitable Wavelength for Monitoring Color Removal

The crude fatty acid solution was a strong dark brown color which

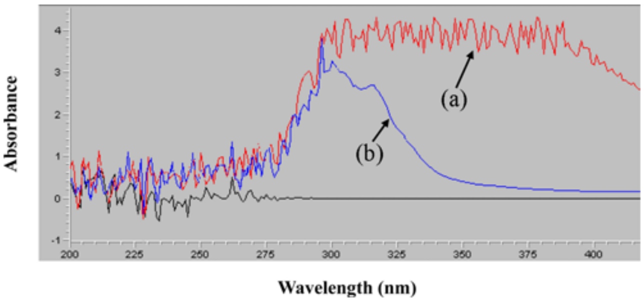

Fig. 1. UV/Visible spectra of crude fatty acids solution before (a) and after (b) decolorization. Crude fatty acids were recovered from crude glycerol and diluted in hexane. Acid-activated clay (2 g) was added to the crude fatty acid solution (10 mL) and the slurry was shaken at 30 °C and 100 rpm for 30 min.

disappeared when the sample was treated with clay. Treated and untreated samples were scanned on a UV-visible spectrophotometer. As shown in Fig. 1, the crude fatty acid solution demonstrated strong absorption at wavelengths of 300-390 nm, whereas peaks corresponding to wavelengths >365 nm disappeared after decolorization. Considering that most peaks appear at 230-270 nm in purified fatty acids [14,15], this result clearly indicates that colored impurities existing in the crude fatty acids contributed to absorb light at wavelengths >365 nm and that clay can remove them effectively by adsorption. These impurities may be non-volatile decomposed compounds such as oxidized triacylglycerols and free fatty acids, which are generated during the biodiesel production process [16], and they were not separated when the crude glycerol was recovered. The color intensity of the crude fatty acid solution decreased when it was diluted with hexane. Notably, a plot of absorbance at 375 nm vs. dilution factor provided a straight line (data not shown). This result clearly indicates that removing color can be monitored by measuring absorbance at this wavelength.

2. Effect of Temperature and Contact Time on Decolorization

The efficiency of decolorizing a crude fatty acid solution can be influenced by several factors such as bleaching temperature, contact time, concentrations of color impurities and crude fatty acids, and the amount of clay. In preliminary experiments, the effect of temperature and contact time was investigated. Generally, oil bleaching processes using adsorbents are carried out at bleaching temperatures of 80-120 °C and contact times of 20-40 min depending on the type of oil and adsorbent [9,17]. Room temperature (30 °C) and 70 °C were selected as low and high level. Higher temperature could not be tested because boiling point of solvent (hexane) is close to 70 °C. Conventional (30 min) and longer contact time (120 min) was selected as low and high level.

Successful decolorization (≥90%) was achieved in 30 min when the crude fatty acid solution was treated at 30 °C (Table 2). No additional improvement occurred despite increasing the temperature or extending the contact time. In fact, the temperature effect during the oil bleaching process is related to oil viscosity. When oil viscosity is high, transport mechanisms slow down due to decreased diffusion rates of the colored materials to the adsorbent surfaces. Therefore, the time required to reach adsorption equilibrium decreases as temperature increases [18,19]. Another possibility is that an increase in adsorption rate with temperature can be attributed to the activation of more adsorption sites by heat [20]. The former suggestion is more plausible for decolorization of the crude fatty acid solution with clay. However, the viscosity of this crude fatty acid solution was much lower than that of oils for refining in conventional processes because the concentrated fatty acid solution was suitably diluted with hexane. Therefore, contact between the color impurities and clay was not prevented even at 30 °C. This result indicates that bleaching temperature and contact time were not critical factors for removing color impurities in a crude fatty acid solution with clay in this study. Therefore, other factors were further optimized to achieve complete decolorization at 30 °C for 30 min.

Table 2. Effect of temperature and contact time on decolorization[a]

Time (min)	Decolorization efficiency (%)[b]	
	30 °C	70 °C
30	90.0±1.6	87.9±1.9
120	87.9±2.9	84.5±0.7

[a] Ten mL fatty acid solution (OD_{375}=38) and 2 g acid-activated clay were mixed for decolorization

[b] Data are shown as removal (%)±S.D.

3. Determining the Optimal Amount of Clay and Initial Color Intensity of the Crude Fatty Acid Solution

Although clay is a good adsorbent for removing color impurities from a crude fatty acid solution, it has a limited adsorption capacity for these impurities. Therefore, the optimum ratio between the amount of clay required and concentration of impurities to be removed should be determined to achieve maximum decolorization. These two factors were therefore optimized in experiments according to CCD, in which incubation temperature and contact time were fixed at 30 °C

Table 3. Experimental runs of central composite design for decolorization of the crude fatty acid solution

Symbol	X_1 (Clay, g)		X_2[a] (OD_{375} of fatty acids solution)		Decolorization efficiency (%)	
Run	Coded	Uncoded	Coded	Uncoded	Exp.	Calc.
1	+1	8	−1	20	99.4±0.1	98.8
2	+1	8	+1	80	97.6±0.1	98.4
3	−1	2	+1	80	83.5±0.2	83.8
4	−1	2	−1	20	98.9±0.1	97.8
5	1.414	9.2	0	50	99.3±0.1	99.0
6	0	5	1.414	92.4	91.6±0.4	90.8
7	−1.414	0.76	0	50	87.5±0.2	88.0
8	0	5	−1.414	7.6	99.9±0.0	100
9	0	5	0	50	98.8	98.8
10	0	5	0	50	98.8	98.8
11	0	5	0	50	98.6	98.8
12	0	5	0	50	98.8	98.8
13	0	5	0	50	98.7	98.8
14	0	5	0	50	98.8	98.8

[a] X_2 is the relative concentration of color impurities in the initial crude fatty acid solution and is represented as OD_{375}

Table 4. Estimated regression coefficients and significance test for the second-order model

Parameter	Coefficient estimate	Standard error	t-Value	Prob>|t|
Intercept	98.7500	0.3048	323.96	<.0001
X_1	3.9112	0.2640	14.82	<.0001
X_2	−3.6176	0.2640	−13.70	<.0001
$X_1{*}X_1$	−2.6067	0.2748	−9.49	<.0001
$X_2{*}X_2$	−1.4314	0.2748	−5.21	0.0008
$X_1{*}X_2$	3.4000	0.3733	9.11	<.0001

Table 5. Analysis of variance for decolorization using acid-activated clay[a]

Source	Degree of freedom	Sum of squares	Mean square	F value	Prob>F
Model	5	334.6944	66.9389	120.0697	<0.0001
Error	8	4.4599	0.5575	-	-
Total	13	339.1543		-	-

[a] $R^2=0.987$

and 30 min, respectively. Fatty acid solutions with different concentrations of color impurities were prepared by diluting the concentrated dark colored fatty acid solution with hexane. As mentioned above, relative concentrations of color impurities can be monitored by measuring OD_{375}.

The design matrix and corresponding results from separate experiments are shown in Table 3. A regression analysis of the data performed using an SAS program generated a second-order polynomial equation. The statistical significance of the model equation was evaluated by analysis of variance (ANOVA), which indicated that the model terms, X_1, X_2, X_1X_2, X_1^2, and X_2^2 were significant ($P<0.005$), as shown in Table 4. The overall second-order polynomial equation for decolorization was:

$$Y=98.750+3.911X_1-3.618\,X_2-2.607\,X_1^2-1.431X_2^2+3.400\,X_1\,X_2$$

where Y is decolorization efficiency (%), and X_1 and X_2 are the coded values of the amount of clay and the OD_{375} value of the fatty acid solution, respectively. Table 5 shows the verification of the model based on the results of an F-test and ANOVA. The value of the $P_{model}>F$ was less than 0.0001, indicating that the results produced by the model were significant. The coefficient of determination (R^2) was 0.987 when goodness of fit of the model was tested, which indicated that only 1.3% of the total variation was by chance and, thus, was not explained by the model [21,22]. The accuracy of the model was verified by the data in Table 3, which showed that the observed and predicted values for decolorization were almost equal. The signs of the coefficients of the factors in the model equation indicated their relative effects on decolorization efficiency. The positive sign for the amount of clay (X_1) indicated that a higher response could be obtained if the applied amount of clay was greater than that of the center point, whereas the negative sign for OD_{375} (X_2) indicated the opposite [21,22]. The model equation showed the presence of the interaction between the two factors. Therefore, the model equation suggests that maximum decolorization can be obtained using a higher amount of clay than that of the center point while also using a fatty acid solution with OD_{375} that is lower than the center point. A contour plot was drawn based on the model equation, which clearly shows the effect of the two factors on decolorization efficiency (Fig. 2). This plot indicates that the color was not removed completely even though the maximum amount of clay was loaded to treat the fatty acid solution with OD_{375} higher than the center points. In contrast, many experimental conditions can be used to achieve complete decolorization (100%) depending on the OD_{375} of the fatty acid solution if its OD_{375} is less than that of the center point following dilution with hexane. It is necessary to consider the operational and economic aspects to choose a suitable operation point to achieve complete decolorization. A small amount of clay is required for decolorizing a weakly colored fatty acid solution (low OD_{375}), but a large volume of hexane is required to dilute fatty acid solution. In contrast, a larger amount of clay is required for decolorizing a strongly colored fatty acid solution (high OD_{375}) although the required volume of hexane is smaller than formal one. Dilution of crude fatty acids with hexane leads to concomitant decrease in fatty acid concentration as well as OD_{375}, thereby reducing solution viscosity. Furthermore, it is easier to separate clay from a slurry mixture by centrifugation or filtration if the crude fatty acid solution is less viscous. Clay will be disposed of as waste after use but hexane can be recovered for reuse by distillation. Considering the economic, environmental, and operational aspects, use of the least amount of clay rather than saving hexane is a better approach. Therefore, we selected the point (−0.5, −1.0) at which complete decolorization was observed on the contour plot. This condition corresponded to 3.5 g clay and starting with a crude fatty acid solution of modest color ($OD_{375}=20$). When, triplicate experiments were performed under these conditions, the observed decolorization was 99.7±0.1%, which corresponded fairly well to the predicted value. Samples with and without decolorization are compared in Fig. 3. The results clearly indicate that acid-activated clay can completely remove color impurities in a crude

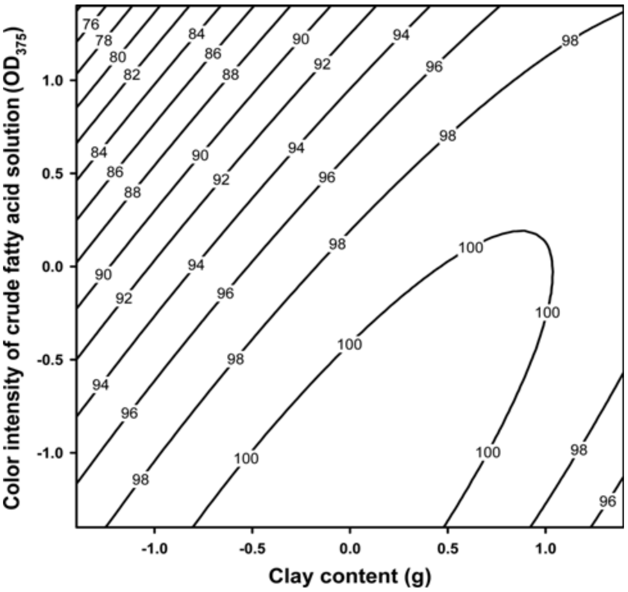

Fig. 2. Contour plot of decolorization of crude fatty acid solution in response to varying the amount of acid-activated clay and color intensity of the initial crude acid solution.

(a) (b)

Fig. 3. Crude fatty acid solution before (a) and after (b) decolorization by acid-activated clay under optimal conditions.

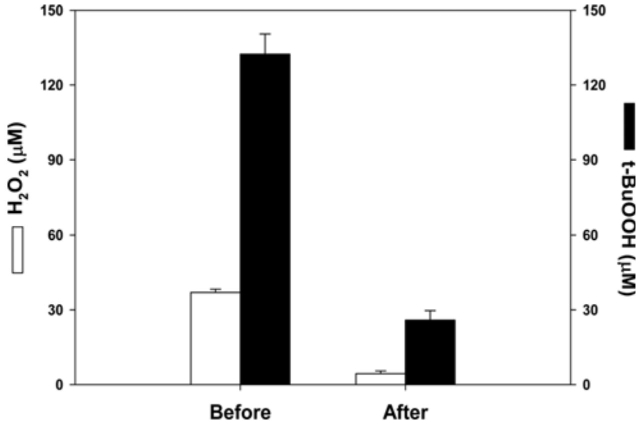

Fig. 4. Removal of peroxides from crude fatty acids during decolorization using acid-activated clay.

fatty acid solution under optimal condition.

4. Removal of Peroxides during Decolorization

Some biodiesels are produced by transesterification of spent vegetable oils, which may contain oxidized products such as peroxides. More peroxides are generated during biodiesel production due to the high temperature reaction. Therefore, crude fatty acids derived from this process may contain peroxides. Contact of peroxides with the skin can cause severe skin damage or premature skin aging [23-25]. Furthermore, they cause damage to the cellular molecules such as DNA, proteins, lipids [25] even at low concentration if passed through a cytoplasmic membrane. These highly reactive materials may also cause side reactions during chemical processes. Therefore, crude fatty acids cannot be used as cosmetic ingredients or raw materials for chemical industries without removing these toxic compounds. Thus, the ability of clay to remove peroxides was evaluated.

Decolorization was performed under optimal conditions; 3.5 g of clay was added to 10 mL of fatty acid solution (OD_{375}=20), and the mixture was incubated at 30 °C for 30 min. Then, the amount of peroxides was compared in the fatty acid solution before and after decolorization. H_2O_2 and t-BuOOH were used as representative inorganic and organic peroxides, respectively. As expected, a high amount of peroxides was detected in the fatty acid solution, and t-BuOOH (132 mM) accumulated much more than H_2O_2 (37 mM), as shown in Fig. 4. Notably, H_2O_2 and t-BuOOH were 4.4 μM and 26 μM, respectively in the decolored sample, indicating that more than 80% of the peroxides were removed. This result clearly indicates that clay can remove peroxides concomitantly as well as color impurities from a crude fatty acid solution. The destruction of peroxides in oils by acid-activated clays has been demonstrated in previous reports [17,18,26,27].

5. Loss of Fatty Acids during Decolorization

Clay is an adsorbent, so we wondered whether it adsorbed not only color impurities but also fatty acids. To answer this question, gas chromatography analysis was performed to analyze the fatty acids in the samples. By comparing with the chromatogram of an authentic sample, we discovered that palmitic acid (C16:0), oleic acid (C18:1), and linoleic acid (C18:2) were the predominant crude fatty acids (>79%). Therefore, peak areas of these components in samples before and after decolorization were compared to estimate the relative changes in these fatty acids in the sample after decolorization. As shown in Table 6, the amount of these fatty acids decreased by approximately 37% after decolorization. This result indicates that clay also removes fatty acids. Adsorption of free fatty acids onto acid-activated clay has been demonstrated, and adsorption capacity varies depending on the acid treatment conditions [18,28,29]. However, it appeared that clay removed the color impurities and toxic peroxides much more selectively than the fatty acids because they were almost completely removed, whereas only one-third of the fatty acids were removed.

6. Reusability of Clay

It is more cost-effective and minimizes waste generation if clay is reused. Accordingly, repeated decolorization cycles were performed using clay to evaluate the reusability of the clay. Decolorization efficiency (%) decreased rapidly as the cycles were repeated and, thus, efficiency was only 17.6% after the third cycle (Fig. 5). It was suspected that the loss of decolorization capacity was due to saturation of the adsorption sites on the clay with organic materials, including color impurities during the recycling process. Therefore, clay was calcined at 600 °C for 6 h to burn these organic materials off after every cycle, and then reusability was evaluated in the next

Table 6. Change in fatty acid concentration during decolorization

Fatty acid	Areas		Change (%)[a]
	Starting sample	Decolored sample	
Palmitic acid (C16:0)	1496.2±90.8	945.9±28.3	−37±3.7
Oleic acid (C18:1)	2722.0±495.9	1672.6±35.3	−37±11.5
Linoleic acid (C18:2)	3652.0±228.3	2300.7±92.5	−37±3.8

[a] Change (%)=(area of starting sample−area of decolored sample)×100/area of starting sample

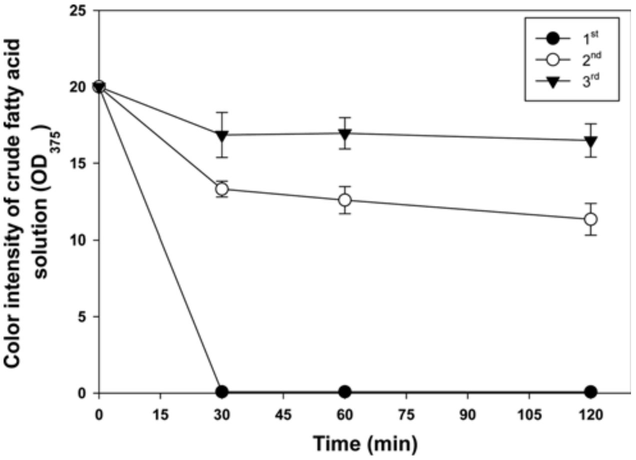

Fig. 5. Decrease in the performance of acid-activated clay following repeated use for decolorizing a crude fatty acid solution.

Table 7. Performance of calcined clay after use in every cycle[a]

Cycles	Color intensity of fatty acid solution (OD_{375})		Decolorization efficiency (%)[b]
	Before	After	
1st	20	0.10±0.02	99.5±0.1
2nd	20	0.16±0.05	99.2±0.3
3rd	20	0.23±0.02	98.9±0.1
4th	20	0.13±0.01	99.3±0.05
5th	20	0.16±0.02	99.2±0.1

[a] Clay was calcined after use in each step

[b] Data are shown as removal (%)±S.D.

cycle. Notably, 99% of decolorization was maintained with no decrease during five repeated cycles, as shown in Table 7. This indicates that the clay could be used repeatedly to remove color impurities with no loss in performance if adsorbed materials are removed by burning. Decolorization efficiency of regenerated clay increases with calcination temperature; 90% decolorization was attained in 15 min with spent clay calcined at 600 °C, whereas decolorization efficiency was lower with spent clay calcined at <500 °C [30]. The optimal calcination temperature is 500 °C to regenerate spent earth to decolorize edible oil, and this temperature corresponds to the beginning of carbonization of organic matter contained in the spent clay [31]. Sintering or destructuring of the clay may occur if spent clay is calcined at >600 °C, leading to lower performance. Taken together, calcination at 600 °C is suitable for regenerating clay for repeated use. Generally, clay from the vegetable oil refining industry is recycled through a calcination process at temperatures of 300-800 °C, followed in some cases by activation with acids [30-32]. However, different from the oil refining process, a further acid activation step was not necessary to regenerate clay in this study and this was probably because fatty acids formed an acidic environment around the clay, which may have contributed to activate the clay.

CONCLUSIONS

Dark-brown colored crude fatty acids were recovered from crude glycerol derived from the biodiesel production process. Color impurities in the fatty acids were removed by acid-activated clay. Bleaching temperature and contact time had no significant influence on decolorization of the crude fatty acids. An optimization study was performed using CCD to determine the minimal amount of clay to achieve complete decolorization. Almost complete decolorization (99.7%) was achieved in 30 min at room temperature when 3.5 g clay and a crude fatty acid solution with modest color (OD_{373}=20) were applied. Notably, more than 80% of the peroxides were concomitantly removed during decolorization under these conditions. We also showed that clay can be reused if it is regenerated by burning adsorbed materials after use. This is the first demonstration of decolorizing crude fatty acids recovered from crude glycerol. Our results will contribute to promote biodiesel production by reducing cost.

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REFERENCES

1. J. C. Thomson and B. B. He, *Appl. Eng. Agr.*, **22**, 261 (2006).
2. F. Yang, M. A. Hanna and R. Sun, *Biotechnol. Biofuels*, **5**, 13 (2012).
3. D. J. Pyle, R. A. Garcia and Z. Wen, *J. Agric. Food Chem.*, **56**, 3933 (2008).
4. K. P. Venkataramanan, J. J. Boatman, Y. Kurniawan, K. A. Taconi, G. D. Bothun and C. Scholz, *Appl. Microbiol. Biotechnol.*, **93**, 1325 (2012).
5. A. M. R. Alvarez and M. L. G. Rodriguez, *Fasc.*, **51**, 74 (2000).
6. S. A. Ibrahim and S. K. Li, *Pharm. Res.*, **27**, 115 (2010).
7. P. Maki-Arvela, J. Kuusisto, E. M. Sevilla, I. Simakova, J.-P. Mikkola, J. Myllyoja, T. Salmi and D. Y. Murzin, *Appl. Catal. A Gen.*, **345**, 201 (2008).
8. P. Gonus and H.-J. Wille, US Patent, 5,401,862 (1995).
9. N. Worasith, B. A. Goodman, N. Jeyashoke and P. Thiravetyan, *J. Am. Oil Chem. Soc.*, **88**, 2005 (2011).
10. B. Makhoukhi, M. A. Didi, D. Villemin and A. Azzouz, *Grasas Y. Aceites*, **60**, 343 (2009).
11. F. Hussin, M. K. Aroua and W. M. A. W. Daud, *Chem. Eng. J.*, **170**, 90 (2011).
12. U. N. Wanasundara and F. Shahidi, *Food Chem.*, **65**, 41 (1999).
13. S. Hu, X. Lu, C. Wan and Y. Li, *J. Agric. Food Chem.*, **60**, 5915 (2012).
14. R. L. Arudi, M. W. Sutherland and B. H. J. Bielski, *J. Lipid Res.*, **24**, 485 (1983).
15. R. T. Holman, W. O. Lundberg, W. M. Lauer and G. O. Burr, *J. Am. Chem. Soc.*, **67**,1285 (1945).
16. C. Mohammed, Y. Alhassan, G. I. Yargamji, S. Garba, Z. Bello and A. I. Ifeyinwa, *J. Basic. Appl. Chem.*, **1**, 80 (2011).
17. M. A. Usman, V. I. Ekwueme, T. O. Alaje and A. O. Mohammed, *ISRM Ceramics*, 5 (2012).

18. N.-A. B. Joy, K. Richard and N. J. Pierre, *J. Appl. Sci.*, **7**, 2462 (2007).
19. T. Langmaack and R. Eggers, *Eur. J. Lipid Sci. Technol.*, **104**, 98 (2002).
20. E. C. Achife and J. Ibemesi, *J. Am. Oil Chem. Soc.*, **66**, 247 (1989).
21. S. K. Ahuja, G. M. Ferreira and A. R. Moreira, *Biotechnol. Bioeng.*, **85**, 666 (2004).
22. L. Huang, Z. Lu, Y. Yuan, F. Lu and X. Bie, *J. Ind. Microbiol. Biotechnol.*, **33**, 55 (2006).
23. A. J. P. Klein-Szanto and T. J. Slaga, *J. Invest. Dermatol.*, **79**, 30 (1982).
24. R. Ganceviciene, A. I. Liakou, A. Theodoridis, E. Makrantonaki and C. C. Zouboulis, *Dermato-Endocrinology*, **4**, 308 (2012).
25. J. A. Lewis II, J. C. Dinardo and D. H. McDaniel, *Cosmet. Dermatol.*, **22**, 576 (2009).
26. L. Meesuk and S. Seammai, *ScienceAsia.*, **36**, 33 (2010).
27. P. Falaras, I. Kovanis, F. Lezou and G. Seiragakis, *Clay Minerals*, **34**, 221 (1999).
28. A. Sari and O. Ipyldak, *Bull. Chem. Soc. Ethiop.*, **20**, 259 (2006).
29. K. R. Rogan, *Colloid. Polym. Sci.*, **272**, 82 (1994).
30. E. L. Foletto, C. C. A. Alves, L. R. Sganzerla and L. M. Porto, *Latin American Applied Research*, **32**, 205 (2002).
31. A. Boukerroui and M.-S. Ouali, *J. Chem. Technol. Biotechnol.*, **75**, 773 (2000).
32. K.-S. Low, C.-K. Lee and L.-Y. Kong, *J. Chem. Technol. Biotechnol.*, **72**, 67 (1998).