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Transesterification of Waste Cooking Sunflower Oil by Porcine Pancreas Lipase Using Response Surface Methodology for Biodiesel Production

Soraya Ebrahimi[1], Ghasem D. Najafpour[1], Fatemeh Ardestani*[2]

1-Faculty of Chemical Engineering, Babol Noshirvani University of Technology, Babol, Iran.

2- Department of Chemical Engineering, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran.

Abstract

Background and Objective: Biodiesel production from recycled vegetable oils is considered as an economically acceptable alternative for fossil fuels in the recent years. In this work, porcine pancreas lipase as an active catalyst in transesterification reaction of waste cooking sunflower oil with methanol for biodiesel production was used.

Material and Methods: In order to define optimum process parameters and predict the best results, response surface methodology and the central composite design was performed. The effects of methanol to oil molar ratio, lipase concentration and reaction temperature on transesterification were investigated. Biodiesel production was carried out in 25 ml shake flasks at 180 rpm for 72 h.

Results and Conclusion: Under optimal conditions, the biodiesel yield was 75% which was nearly consistent with the predicted yield of 76%. At optimal conditions the molar ratio of methanol to oil, reaction temperature, and lipase percent were determined as 3:1, 44°C and 4.4%, respectively. Due to relatively high obtained yield, biodiesel production from waste cooking sunflower oil has provided a sound environmental and commercial process.

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***Corresponding author:**

Fatemeh Ardestani,
Department of Chemical Engineering, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran.

Tel: +98-1142155025
Fax: +98-1142240091
E-mail: f.ardestani@qaemiau.ac.ir

1. Introduction

Today, around the world, many researchers have focused on biodiesel production as a renewable energy source and a suitable alternative case for current petroleum derived fuels [1]. In terms of chemical structure, biodiesel is a mono-alkyl ester of long chain fatty acids, meets the specifications of ASTM D 6751 with numerous advantages compared to petro-diesel such as biodegradability, lack of sulfur, lower emissions, environmental friendly and positive energy balance and renewability [2,3]. Biodiesel is often produced via transesterification of vegetable oils, waste cooking oils or animal fats with mono-alcohols, carried out through enzymatic catalysts (Figure. 1). Its production from waste cooking oils, inedible vegetable oils and waste coffee beans has been noted by many researches recently [4-6].

Sunflower oil is a triglyceride derived from glycerol having three units of fatty acids mostly includes 30% of monounsaturated omega-9 oleic acid and 59% of polyunsaturated omega-6 linoleic acid [6]. Every year, a remarkable amount of sunflower oil cooking waste contamination is produced by food processing factories and restaurants as well as cooking appliances. Using this waste material in biodiesel production not only protects the water resources but also brings some noteworthy economic benefits [7]. Lipase is of the most attractive biocatalysts used for the esterification reaction due to its ability to carry out bioconversion of all mono, di- and triglycerides [8,9]. Porcine pancreas lipase (triacylglycerol ester hydrolase, EC 3.1.1.3) has major advantages including cost efficiency, sufficient availability, high thermal resistance, and retaining activity in dry condition [10].

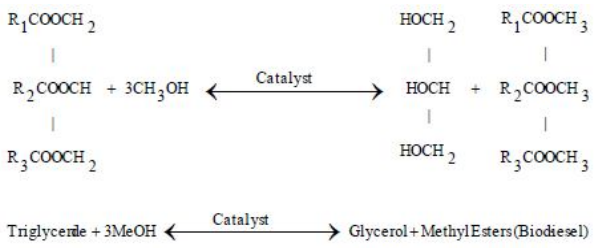

Figure 1. Biodiesel production using transesterification reaction

Determination of an appropriate reaction temperature, molar ratio of methanol to oil and biocatalyst concentration are essential to achieve high tranesterification yield [11]. Reaction conversion is strongly affected by the molar ratio of methanol to oil. Based on the reaction stoichiometry, one molecule of triglyceride reacts with three molecules of methanol, but excess methanol boost the reaction yield [12]. Many ongoing researches are focused to find out the optimum reaction parameters and conditions to reach the highest quality biodiesel [13].

Salis et al. studied biodiesel production from triolein oil and butanol as a short chain alcohol by different commercial immobilized lipases in solvent free conditions and after 6 h, found 100% conversion of butanol using *Pseudomonas cepacia* lipase at the optimal conditions [14]. Sanchez and Vasudevan determined the best molar ratio of methanol to oil at 3:1 and reaction temperature of 60^{o}C. They also demonstrated independency of biodiesel production yield from initial enzyme concentrations of > 500 U ml^{-1} triolein oil [15]. A one step transesterification of sunflower oil by free lipase from *Thermomyces* (*T.*) *lanuginosus* obtained a conversion of 95% [16]. Also a yield of 46% was obtained for biodiesel production from waste cooking oil with high free fatty acid contents in the presence of zeolite-based catalyst at 70^{o}C and methanol to oil molar ratio of 5:1 for reaction time of 6 h [17] and a conversion of 96.66% in two-stage process for biodiesel production from waste cooking oil and methanol [18]. A biodiesel yield of 69% was reported by Choi et al. from *Scenedesmus* sp. at the optimal condition of 5.46% catalyst (H_2SO_4) and biomass to methanol ratio of 1:22 at 70°C for 10 h reaction time [19].

As listed in Table 1, Portha et al. developed a numerical model to investigate different coupled aspects of biodiesel production process and found 87% conversion rate with molar ratio of methanol to oil equal to 3.6:1 at 1 h reaction time [20]. Wong and Devi reported the best reaction time and temperature of 1 h and 60^{o}C for transesterification of used cooking oil in the presence of KOH as alkaline catalyst [21]. Various biodiesel production yields were reported by different lipase sources and various feedstocks are summarized in Table 1. Some antioxidant agents such as 2, 6-di-tert-butyl-4-methyl-phenol were used to protect biodiesels from oxidative destruction [22]. Many recent researches focused on biodiesel production from microalgae species such as *Chlorella protothecoides* with considerable oil content, high proliferation speed and significant biomass production rate and 97.25% methyl ester yield at optimal condition [23].

In this study, response surface methodology applied in order to define the effects of various parameters such as methanol to oil molar ratio, enzyme concentration, and reaction temperature on transesterification of waste cooking sunflower oil catalyzed by porcine pancreas lipase.

Table 1. Biodiesel production yield in enzymatic transesterification using different types of oils and lipase catalyst

Feedstock	Solvent	Acyl-acceptor	Lipase source	Reaction condition	Biodiesel yield(%)	Reference
Soybean oil	Ionic liquid	Methanol	*Candida antarctica*	40^{o}C for 12 h	80	[28]
Rapeseed oil	Petroleum ether	Methanol	*Candida* sp. 99-125	40^{o}C for 36 h, 180 rpm	83	[29]
Mahua oil	No solvent	Ethanol	*Pseudomonas cepacia*	40^{o}C for 6 h, 200 rpm	96	[30]
Sunflower oil	N-hexane	Ethanol	*Rhizomucor miehei*	40^{o}C for 24 h	79.1	[31]
Jatropha oil	No solvent	Methanol	*Rhizopus oryzae*	30^{o}C for 60 h	80	[32]

2. Materials and Methods

2.1. Materials

Porcine pancreas lipase (250 units/mg protein), methanol, methyl pentadecanoate, methyl oleate, methyl linoleate, methyl stearate and methyl palmitate provided by Sigma-Aldrich company, USA. Waste cooking sunflower oil was collected from household consumers in Qaemshahr city, Iran.

2.2. Experimental design

The percent of biodiesel production from waste cooking sunflower oil in batch system designed by response surface methodology using Design Expert 7.0 (Stat-Ease, Inc, Minneapolis, USA) with three independent reaction variables including reaction temperature (X_1), methanol to oil molar ratio (X_2), and initial weight percentage of lipase (X_3), each one coded at five levels: −2, −1, 0, +1 and +2 (Table 2). This method minimizes the number of experiments as well as reduces systematic errors together with an estimation of the experimental errors. The central composite design applied to evaluate the relationship between independent variables and responses to optimize the reaction parameters with the aim of predicting the best value of responses. Optimized values of the reaction variables determined through solving the regression equation at the desired values of the process responses as the optimization criteria. Probability (P-value) with a 95% confidence level used to confirm or decline the model terms. Experimental results analyzed via the analysis of variance (ANOVA). The simultaneous interaction of the two factors on the responses studied in terms of three dimensional plots.

Table 2. Experimental ranges and levels of the independent reaction variables

Variables	Symbol	Range and levels				
		-2	-1	0	1	2
Reaction temperature (°C)	X_1	25	35	45	55	65
Methanol to oil molar ratio	X_2	1	2	3	4	5
Enzyme dosage (%)	X_3	1	2	3	4	5

Table 3. Designed experiments, actual and predicted biodiesel production yield based on the central composite design

Run no.	Coded values			Biodiesel yield (Y%)	
	X_1	X_2	X_3	Actual	Predicted
1	0	0	2	75.65	73. 86
2	1	1	1	55.79	58.17
3	1	-1	-1	19.47	18.24
4	1	1	-1	21.77	21.91
5	1	-1	1	39.82	42.42
6	0	0	-2	16.06	19.07
7	0	0	0	65.11	65.41
8	-1	-1	-1	45.03	42.69
9	-1	1	-1	22.59	20.03
10	0	0	0	65.98	65.41
11	2	0	0	13.43	11.50
12	-1	1	1	52.07	53.34
13	0	2	0	22.96	22.37
14	0	0	0	69.11	65.41
15	-1	-1	1	64.01	63.92
16	0	-2	0	28.73	29.28
17	0	0	0	65.21	65.41
18	0	0	0	61.92	65.41
19	0	0	0	65.18	65.41
20	-2	0	0	29.236	31.12

2.3. Biodiesel production experiments

All experiments of enzymatic transesterification of waste cooking sunflower oil with methanol carried out in 25 ml flasks. Initially, 2 g oil added to each flask and then, methanol and porcine pancreas lipase added to reaction flask based on the designed procedure. All reaction flasks placed in an incubator shaker (IKA, KS 4000 i control, Germany) at 180 rpm for 72 h. Methanol added in a three-step procedure. In each step one equivalent molar of methanol added at the reaction time of 0, 18 and 36 h. At the end of reaction, lipase has been inactivated by putting the flasks at 90°C water bath for 10 min [9,12].

2.4. Analytical methods

The composition of the produced biodiesel assayed by a Gas Chromatography analyzer (Agilent 9780, USA) equipped with flame ionization detector (FID) and a 19091J-413 HP5 capillary column (30m×0.32 mm×0.25µm). Samples collected at the end of reaction time and centrifuged at 5000 ×g for 10 min. Then for GC analysis, 1 µl of each sample used for injection after methylation. Column temperature kept at 180°C for 10 min. after that the temperature was raised to 280°C with a rate of 5°C per minute and kept for 10 min. Nitrogen used as carrier gas with a flow rate of 7.5 ml min^{-1}. Injector and detector temperatures were 230°C and 280°C, respectively. A calibration curve with R^2=0.997 and a linear equation (Eq. 1) determined based on standard solutions of Methyl pentadecanoate, methyl oleate, methyl linoleate, methyl stearate and methyl palmitate from 400 to 1000 mg l^{-1}. Methyl pentadecanoate was used as standard into each biodiesel sample prior to GC analysis to determine biodiesel production yield based on Eq. 2 [24].

$$Y=0.002X+0.389 \quad \text{Eq. 1}$$

$$\text{Biodiesel yield} = \frac{\text{Area of all FAME}}{\text{Area of reference}} \times \frac{\text{weight of reference}}{\text{weight of biodiesel sample}} \times \frac{\text{weight of biodiesel producted}}{\text{weight of oil used}} \times 100 \quad \text{Eq. 2}$$

3. Results and Discussion

Biodiesel production via transesterification of waste cooking sunflower oil by porcine pancreas lipase was carried out in a batch system. In order to obtain the highest possible yield, reaction parameter optimization were carried out through response surface methodology.

3.1. Biodiesel production yield

Results on biodiesel production yield in CCD experiments based on three independent reaction variables together with the predicted mean and observed responses are presented in Table 3. The experimental data on biodiesel production yield were subjected to multiple regression analysis and the CCD design experimental results matched a full second-order polynomial equation as described at Eq. 3.

$$Y=65.41-4.90X_1-1.73X_2+14.37X_3+6.58X_1X_2+0.74X_1X_3+3.02X_2X_3-11.02X_1^2-9.9X_2^2-4.4X_3^2 \quad \text{Eq. 3}$$

3.2. Results analysis

The ANOVA was performed to evaluate the accuracy of the model (Eq. 3), presented in Table 4. High F-value (154.35) and very low P-value (≤0.0001) indicated the significant capability of the applied model to predict the experimental results and confirmed the models accuracy. Analysis also showed a high significant regression of 0.993 at 95% confidence level. The value of the predicted multiple correlation coefficient (Pred. R^2) was 0.962 that indicated a good accordance with adjusted multiple correlation coefficient (Adj. R^2 = 0.986).

The factors first-order effects, quadratic effects, and interaction effects were specified as significant model terms. The importance of Eq. 3 coefficients, the effects of each independent reaction variables, and the interactions of parameters were investigated on biodiesel production yield through the statistical quantities presented in Table 5.

Table 4. Analysis of variance (ANOVA) for the response surface quadratic model in biodiesel production from waste cooking sunflower oil

Source	Sum of squares	Degrees of freedom	Mean square	F-value	Probability (P) > F
Model	8487.47	9	943.05	154.35	≤0.0001
Residual	34.72	5	6.94	1.32	0.3852
Pure error	61.1	10	6.11		
Total	26.38	5	5.28		

Table 5. Statistical quantities indicative the effects of independent reaction variables on biodiesel production yield

Model term	Coefficient estimate	Standard error	F-value	P-value
Intercept	65.50	0.99		
X_1	-4.90	0.62	62.99	≤ 0.0001
X_2	-1.73	0.62	7.82	0.0189
X_3	13.88	0.62	504.20	≤ 0.0001
X_1X_2	6.58	0.87	56.71	≤ 0.0001
X_1X_3	0.74	0.87	0.71	0.4176
X_2X_3	3.02	0.87	11.94	0.0062
X_1^2	-10.98	0.49	496.12	≤ 0.0001
X_2^2	-9.85	0.49	399.42	≤ 0.0001
X_3^2	-4.85	0.49	96.77	≤ 0.0001

Low amounts of P-value for all reaction variables include reaction temperature, methanol to oil molar ratio and initial weight percentage of lipase indicated that biodiesel production yield is a linear and quadratic function of these independent variables. Results also showed that interaction between reaction temperature and enzyme amount is very negligible. On the other hand, none of these two factors had considerable impact on the other one for the biodiesel production yield. While interaction between reaction temperature and methanol to oil molar ratio as well as the interaction between the methanol to oil molar ratio and enzyme amount were evaluated to be relatively significant.

3.3. The effect of reaction variables on biodiesel production yield

Three-dimensional curves presented in Figures 2 to 4 showed the effects of independent reaction variables on biodiesel production yield and the interactions between these variables. Figure 2 represents the contour and 3-D curve of the effect of reaction temperature and enzyme dosage on biodiesel production yield.

At the initial stage of transesterification reaction, biodiesel production yield had raised with the increase in both reaction temperature and enzyme dosage. But this raising profile was stopped and converted to a descending one at reaction temperatures higher than 43^{o}C and lipase weight ratios more than 3.48 (w w^{-1}). This suggests that enzyme activities gradually decline at temperature above 43^{o}C which led to irreversible inactivation of porcine pancreas lipase in accordance with previous reported data [25].

The similar behavior was observed for the combined effects of methanol to oil molar ratio and enzyme dosage on biodiesel production yield (Figure 3). At first, an increasing trend was recorded for biodiesel production yield but in the following, at methanol to oil molar ratios greater than 3:1 and lipase weight ratios higher than 3.9 (w w^{-1}) converted to a decreasing trend. Thus, it can be concluded that the presence of high amounts of porcine pancreas lipase in transesterification reaction vessel may have a disincentive effect on the reaction rate and led to low biodiesel production yield.

The same phenomenon was recorded for the simultaneous impacts of the reaction temperature and methanol to oil molar ratio on biodiesel production yield (Figure 4). At the reaction temperatures greater than 42^{o}C and methanol to oil molar ratios more than 2.84:1, biodiesel production yield has experienced a decreasing profile. As the previous researches emphasized, type of applied catalysis, oil to solvent ratio, as well as reactor characteristics are the main effective factors for transesterification reaction in biodiesel production process [26,27].

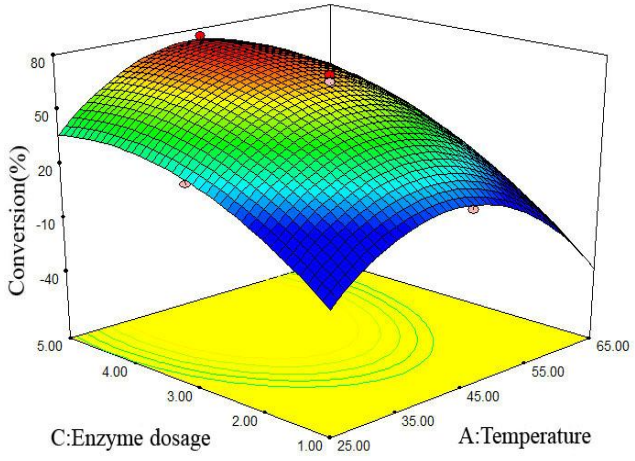

Figure 2. Contour and 3 D diagram of the effect of reaction temperature and enzyme dosage on biodiesel production yield

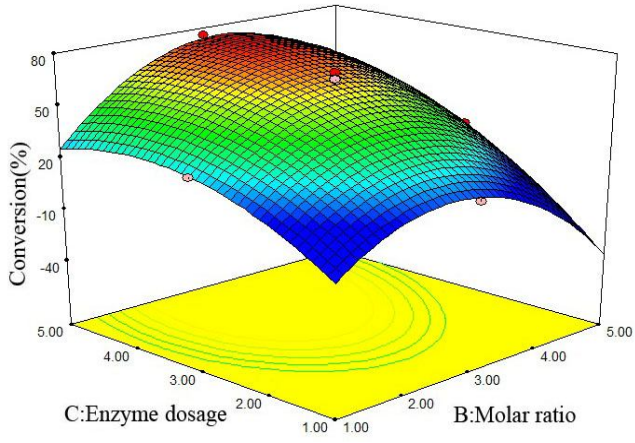

Figure 3. Contour and 3 D diagram of the effect of methanol to oil molar ratio and enzyme dosage on biodiesel production yield

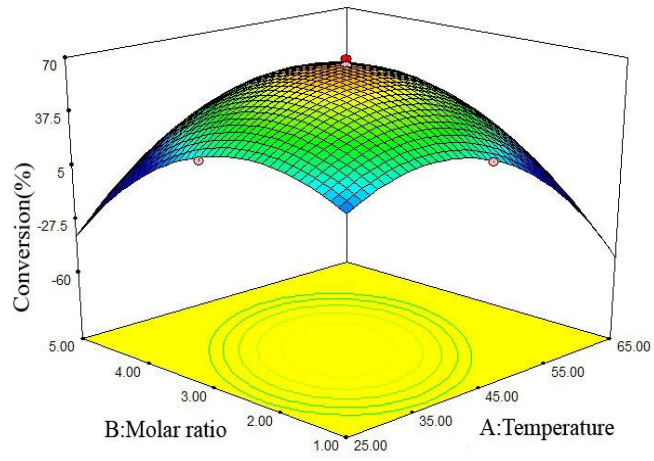

Figure 4. Contour and 3 D diagram of the effect of reaction temperature and methanol to oil molar ratio on biodiesel production yield

3.4. Determination of optimal condition

The optimal values of independent variables were calculated through solving three derived equations obtained from differentiation of Eq. 3.

Accordingly, at optimum condition, the reaction temperature, methanol to oil molar ratio, and initial weight percentage of lipase were determined as 43°C, 3.04:1 and 4.43%, respectively. The statistical predicted biodiesel production yield under optimum reaction condition was calculated equal to 75.8%. An individual experiment was conducted using the optimal reaction variables and the obtained results showed 74.9% biodiesel production yield that is in good consistency with the predicted theoretical values.

The optimum reaction temperature of 43°C obtained for porcine pancreas lipase showed acceptable compliance with the results of Ha et al. for *Candida antarctica* lipase [28], Deng et al. for *Candida* sp. 99-125 lipase [29], Kumari et al. for *Pseudomonas cepacia* lipase [30] and also Soumanou and Bornscheuer for *Rhizomucor miehei* lipase [31]. The obtained biodiesel production yield using waste cooking sunflower oil (75% in the present work) is similar to the results of Ha et al. for soybean oil with a yield of 80% [28], Deng et al. using rapeseed oil and a biodiesel yield of 83% [29], Soumanou and Bornscheuer for sunflower oil and a yield of 79.1% [31], Tamalampudi et al. for jatropha oil with a biodiesel production yield of 80% [32]. However, our obtained yield is less than that of Kumari et al. using mahua oil and ethanol in a 200 h reaction time with biodiesel yield of 96% [30] as well as Salis et al. using triolein oil and butanol with biodiesel production yield of 100% [14].

Also our obtained biodiesel production yield is less than those reported by Budzaki et al. a conversion of 95% for biodiesel production from sunflower oil by free lipase from *Thermomyces lanuginosus* [16] and yet more than Hassani et al. that obtained only a 46% conversion from waste cooking oil transesterification by zeolite-based catalyst at 70°C [17] and Choi et al. with just a 69% biodiesel production yield from *Scenedesmus* sp. at the optimal condition [19].

4. Conclusion

The present study is the first effort to optimize transesterification reaction of waste cooking sunflower oil and methanol in the presence of porcine pancreas lipase using surface response methodology. A significant biodiesel production yield of 80% was obtained under optimal reaction condition. Thus, waste cooking sunflower oil was distinguished to be a suitable feedstock for biodiesel production. It is predicted that in the near future, biodiesel produced from food waste will be a serious alternative to replacing fossil fuels and diesel due to it's environmentally and economically advantages. The highest

activity of porcine pancreas lipase and therefore the best biodiesel production yield was reported at reaction temperature of 43°C. Results also demonstrated that over dosage of enzymes may lead to a decrease in the reaction rate and biodiesel production yield.

5. Acknowledgements

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6. Conflict of Interest

The authors declare that they have no conflict of interest.

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ترانس استری کردن پسماند روغن پخت و پز آفتابگردان توسط لیپاز به‌دست آمده از پانکراس خوک برای تولید بیودیزل با روش سطح پاسخ

ثریا ابراهیمی [1]، قاسم نجف پور [1]، فاطمه اردستانی [2*]

1- دانشکده مهندسی شیمی، دانشگاه صنعتی بابل نوشیروانی، بابل، ایران.
2- گروه مهندسی شیمی، واحد قائمشهر، دانشگاه آزاد اسلامی، قائمشهر، ایران.

چکیده

سابقه و هدف: در سال‌های اخیر، تولید بیودیزل از روغن‌های گیاهی بازیافتی به عنوان یک جایگزین توجیه‌پذیر اقتصادی برای سوخت‌های فسیلی مورد توجه قرار گرفته است. در این تحقیق، آنزیم لیپاز به‌دست آمده از پانکراس خوک به عنوان یک کاتالیزور فعال برای انجام واکنش ترانس استری کردن پسماند روغن پخت و پز آفتابگردان با متانول به منظور تولید بیودیزل مورد استفاده قرار گرفت.

مواد و روش‌ها: به منظور تعیین پارامترهای بهینه فرایند و پیش‌بینی بهترین نتایج، روش پاسخ سطحی و طرح کامپوزیت مرکزی اجرا شد. اثرات نسبت مولی متانول به روغن، غلظت لیپاز و دمای واکنش بر ترانس استری کردن بررسی گردید. تولید بیودیزل در ارلن‌های 25 میلی لیتری و سرعت همزدن 180 دور در دقیقه به مدت 72 ساعت انجام شد.

یافته‌ها و نتیجه‌گیری: تحت شرایط بهینه، ، بازده تولید بیودیزل 75 درصد بود که تقریبا با بازده پیش‌بینی شده‌ی 76 درصدی، تطابق داشت. در شرایط بهینه، نسبت مولی متانول به روغن، دمای واکنش و درصد لیپاز به ترتیب برابر با 3 به 1، 44 درجه سانتیگراد و 4/4 درصد تعیین گردید. بازده نسبتا بالای به‌دست آمده در تولید بیودیزل موجب شد که فرایند تولید بیودیزل از پسماند روغن پخت و پز آفتابگردان به عنوان یک فرایند اقتصادی معرفی شود.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ تعارض منافعی وجود ندارد.

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- ترنس استری کردن
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- لیپاز

***نویسنده مسئول**

فاطمه اردستانی، گروه مهندسی شیمی، واحد قائمشهر، دانشگاه آزاد اسلامی، قائمشهر، ایران.
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پست الکترونیک:
f.ardestani@qaemiau.ac.ir