Super Phosphoric Acid Catalyzed Biodiesel Production from Low Cost Feed Stock

Bharatkumar Z. Dholakiya

Department of Applied Chemistry, Sardar Vallabhbhai National Institute of Technology (SVNIT), Ichhanath, Surat, Gujarat, India

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ABSTRACT

The conventional process for producing biodiesel is base catalyst and requires anhydrous condition and feed stock with low levels of free fatty acid (FFA). Basic catalyst gives the higher reaction rate than acid catalyst. However, the basic catalysts involved soap formation of free fatty acid leading to the deactivation of catalyst and high production cost. Inexpensive feed stocks containing high levels of free fatty acid cannot be directly used with the base catalyst. This work deals with the synthesis of biodiesel from high free fatty acid containing crude degummed cotton seed oil (CDGCSO), using 5 wt % (weight of the oil) super phosphoric acid (SPA) as catalyst and evaluating the effect of the molar ratio (oil : alcohol) on percentage conversion. Molar ratio 1:10 showed greater yield. The acid catalysts do not form the soap and can simultaneously conduct esterification and transesterification of free fatty acid and oil to biodiesel. However, they are slower and necessitate higher reaction temperatures. Nonetheless, acid-catalyzed processes could produce biodiesel from low-cost feed stocks and lowering the cost of production.

Keywords: Super Phosphoric acid, FFA, low cost feed stock, CDGCSO, Biodiesel.

INTRODUCTION

Biodiesel is a nonpetroleum-based fuel that consists of alkyl esters derived from either the transesterification of triglycerides (TGs) or the esterification of free fatty acids (FFAs) with alcohols [1]. It is an alternative to petroleum diesel for reducing emissions of gaseous pollutants such as CO, SOx, particulate matters and organic compounds [2, 3]. The flow and combustion properties of biodiesel are similar to petroleum-based diesel and thus, can be used either as a substitute for diesel fuel or more commonly in fuel blends [4]. It is a clean burning fuel which is non-toxic, biodegradable and considered as the fuel of the future.

Commonly, biodiesel is prepared from TG sources such as vegetable oils, animal fats and waste greases. There are several routes to obtain biodiesel from various feed stocks. But the most

common method is transesterification [5]-[8] in which according to stoichiometry, 1 mol of TG reacts with 3 mol of alcohol in presence of a strong catalyst (acid, base or enzyme), producing a mixture of biodiesel (fatty acid alkyl ester) and glycerol (Scheme-1) [9, 10].
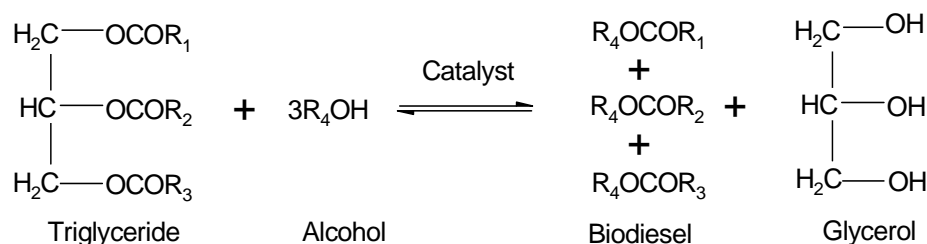
Scheme 1 Transesterification of triglyceride (TG) to Biodiesel.

The transesterification reaction requires a catalyst in order to obtain reasonable conversion rates. The nature of the catalyst is fundamental since it determines the compositional limits that the feed stock must conform to. Furthermore, the reaction conditions and post-separation steps are predetermined by the nature of the catalyst used. Currently most biodiesel is prepared using base catalyst, such as sodium and potassium methoxides and hydroxides. Even though transesterification is feasible using base catalyst, the overall base catalyzed process suffers from serious limitations that translate into high production costs for biodiesel. Strict feedstock specifications are a main issue with this process [2]. The total FFA content associated with the feedstock must not exceed 0.5 wt% in case of base catalyzed process. Otherwise, soap formation seriously hinders the production of fuel grade biodiesel [1, 11, 12]. Soap forms when the base catalyst reacts with FFAs in the feed stocks (Scheme 2a). Soap production gives rise to the formation of gels, increases viscosity and greatly increases product separation cost [9]. The alcohol and catalyst must also comply with rigorous specifications. The alcohol as well as the catalyst must be essentially anhydrous (total water content must be 0.1-0.3 wt% or less) [13]. This is required since it is assumed that the presence of water in the feedstock promoted hydrolysis of the alkyl ester to FFA (Scheme 2b) and consequently, soap formation.
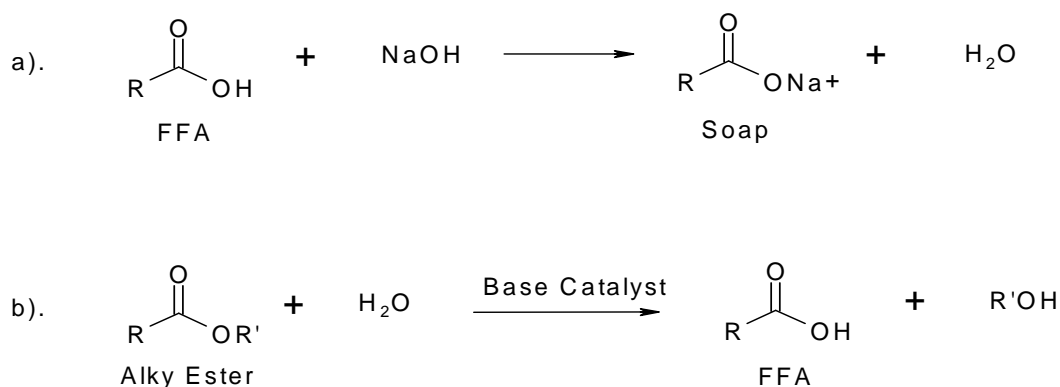
Scheme 2 (a) Base catalyst reaction with FFAs to produce soap and water (b) Water promotes the formation of FFAs.

To conform to such demanding feedstock specifications necessitates use of highly refined vegetable oil whose price can account for 60-75% of the final cost of biodiesel [14]. Other less expensive sources of TG feed stocks such as crude oil, waste oil, and yellow greases can be used to counteract the high price tag associated with biodiesel produced from refined oils using acid catalyzed system. The type of feed stock generally selects the nature of catalyst. If the FFA content is high, acid catalyzed esterification followed by transesterification is used. If FFA

content is low the base catalyzed transesterification is most desirable and is relatively faster than acid catalyzed transesterfication [15]. For acid catalyzed system, sulfuric acid has been the most investigated catalyst, but other acids, such as HCl, BF_3, H_3PO_4 and organic sulphonic acids have also been used by different researches [16]. The influence of process variables on the acid-catalyzed transesterification reaction has been reported in several studies [17]-[21]. Freedman et al. [17] and Canakci and Van Gerpen [18] examined the effect of the alcohol type on the acid-catalyzed transesterification of soybean oil at temperatures just below the boiling points of the alcohols. The results indicated that the effect of the reaction temperature, rather than the type of alcohol used, dominates the rate of the reaction and dictates the time required to achieve complete ester conversion. Canakci and Van Gerpen [18] showed that the ester conversion increased with an increasing temperature, molar ratio of alcohol to oil and acid-catalyst concentration.

Goff et al. [20] conducted a study to investigate the efficiency of different acid catalysts at elevated temperature under different operating conditions and determined that H_2SO_4 was the most effective catalyst for the transesterification reaction. Freedman et al. [21] investigated the acid-catalyzed butanolysis of soybean oil at an alcohol:oil molar ratio of 30:1 and 1 wt % H_2SO_4 catalyst concentration at different temperatures in the range of 77-117 °C. The results indicated that the complete conversion was achieved in 20 hours at 77 °C and   3 hours at 117 °C. Zheng et al. [19] studied the acid-catalyzed transesterification reaction kinetics of waste frying oil using MeOH:oil molar ratios in the range of 50:1-250:1 and acid-catalyst concentrations ranging from 1.5 to 3.5 mol % (on the basis of the oil) at temperatures of 70 and 80 °C. The results demonstrated that the acid-catalyzed transesterification reaction of waste frying oil in MeOH effectively follows pseudo-first-order reaction kinetics.

The demanding feed stocks specifications for base catalyzed reactions have led researcher to seek catalytic and processing alternatives that could ease this difficulty and lowers the cost of production. Methodologies based on acid catalyzed reactions have the potential to achieve this since acid catalysts do not show measurable susceptibility to FFAs. For this reason the development of acid catalyzed methodologies is the focus of this paper.

The aim of this work is to develop the process by using super phosphoric acid (SPA) catalyst to produce the biodiesel from low cost feed stocks (crude degummed cotton seed oil). A major hurdle towards widespread commercialization is the high price of biodiesel. And hence, an attempt is made to produce low price biodiesel by using crude oil. In this study, CDGCSO containing 5% FFA is chosen as a feedstock for biodiesel production. The effect of varying oil : alcohol molar ratios of 1:40, 1:30, 1:20, 1:10 and 1:5 with catalyst (SPA) amount 5 % of the weight of the oil on the transesterification reaction yield were investigated.

MATERIALS AND METHODS

Reagents and Materials
Crude degummed cotton seed oil obtained Bhavani Oil Mill Limited, Botad, Gujarat, India. Super phosphoric acid and 1-butanol used in the present study were purchased from S. D. Fine Chem. Limited, Vadodara, Gujarat, India.

SPA catalyzed biodiesel production from crude degummed cotton seed oil
SPA catalyzed biodiesel synthesis were performed in a three neck 1000 ml round bottom flask equipped with stirrer, thermometer, water condenser and heating system. The biodiesel synthesis was studied at different oil:1-butanol molar ratios (1:40, 1:30, 1:20, 1:10 and 1:5) under reflux at

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120^OC and with catalyst (SPA) amount 5% of the weight of the oil. Reactants were introduced together with the appropriated catalyst mass and time of reaction was considered when desired temperature (generally 120^OC) was reached. Samples were drawn at two hours interval and percentage conversion was estimated by Gel Permeation Chromatography (GPC). After completion of reaction, excess 1-butanol was completely distilled off and the mixture was carefully transferred to a separating funnel and allowed to settle (Fig. 1). The upper layer consists of biodiesel whereas the lower layer contained glycerin and most of catalyst. The upper layer was purified using hot distilled water and then dried over anhydrous sodium sulfate (Na_2SO_4). Figure 2 shows the washed, dried biodiesel and crude degummed cotton seed oil containing 5 % FFA. In this method, 1.0 gm of anhydrous Na_2SO_4 was taken for 100 ml of biodiesel, stirred for 15 minutes and then was allowed to settled and decanted. The decanted dried biodiesel was filtered with the help of vacuum pump for final removal of solid traces. Finally, biodiesel fuel properties were determined with the help of the standard tests (Table 1).
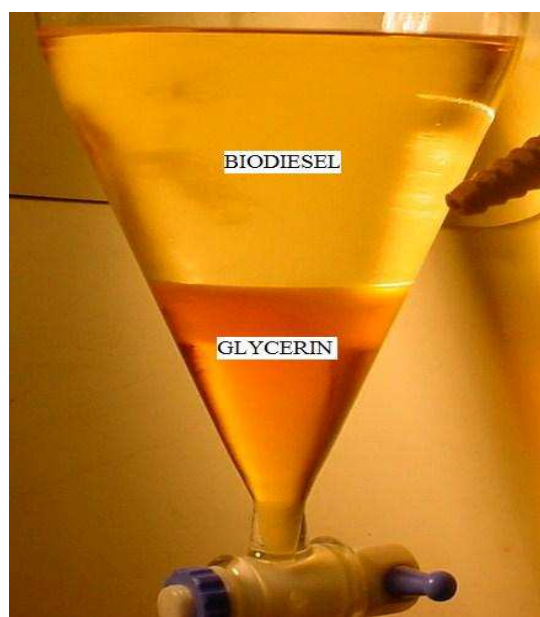
Fig. 1 Separating Funnel:
(1) Upper layer – biodiesel.
(2) Lower layer – glycerin.
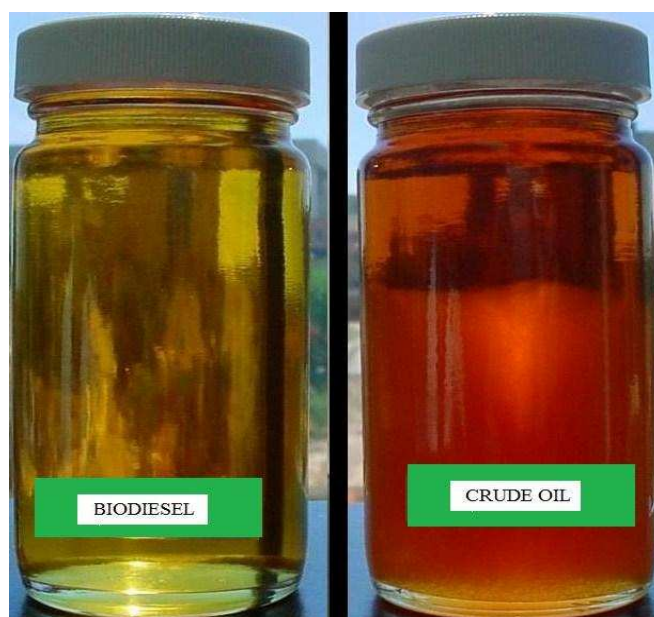
Fig. 2 Biodiesel after washing & drying and
crude degummed cotton seed oil with 5%
FFA.

Experiments were conducted as above to measure the conversion of FFA and TGs in the CDGCSO to corresponding yields of diglycerides (DGs), monoglycerides (MGs) and biodiesel. All experiments were performed at atmospheric pressure till the completion of reaction. Initial analysis of reaction mixture by GPC showed that it contained FFA and TGs (Fig. 3). The analysis of percentage conversion of FFA & TGs to DGs, MGs and biodiesel were carried out at two hours intervals using GPC. At intervals of two hours samples were drawn, filtered through 0.2 µm polytetrafluoroethylene syringe filters. GPC vials containing 0.04 gm of filtered sample were weighed and diluted with THF to make up 20 mg/mL sample solution for GPC analysis.
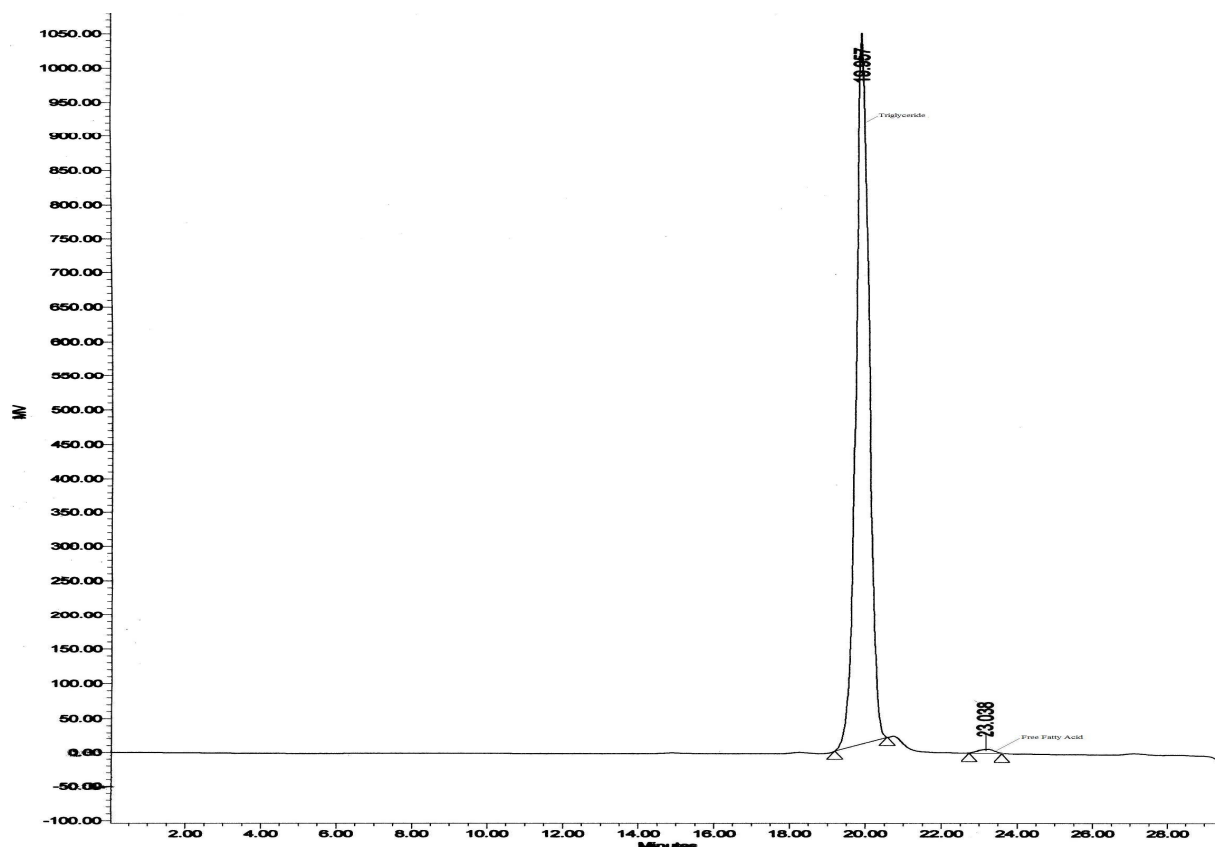
Fig. 3 GPC of CDGCSO.

The GPC of reaction mass was done by using Waters GPC instrument with Waters 600 controller and pumps. The HPLC grade Tetrahydrofuran (THF) was used as a mobile phase at a flow rate of 1.0 ml/min. The GPC system was equipped with three columns: PLgel 50 A^0, Oligopore and PLgel 100 A^0 connected in series. Waters 2410 Refractive Index detector and Waters 2487 Dual λ absorbance detector were used with internal temperature of 35^{0}C for peak detection. The system was operated using Waters Millennium 32 software. The running time required for product characterization was approximately 35 minutes. Calibration curves [22] were generated from the standards: triolein (TG), diolein (DG), monolein (MG), butyl oleate (biodiesel), oleic acid (FFA) and glycerol (GLY). The areas under the peaks in the chromatograms were used to determine the percentage of the constituents (TG, DG, MG, FFA and biodiesel) present in the sample. Figure 4 shows the 99.79% conversion of FFA and TGs to biodiesel at 1:10 oil:1-butanol molar ration after 10 hours.

Fig. 4 GPC of Biodiesel.

R$_1$, R$_2$, R$_3$: Carbon chain of fatty acid
R$_4$: Alkyl group of alcohol
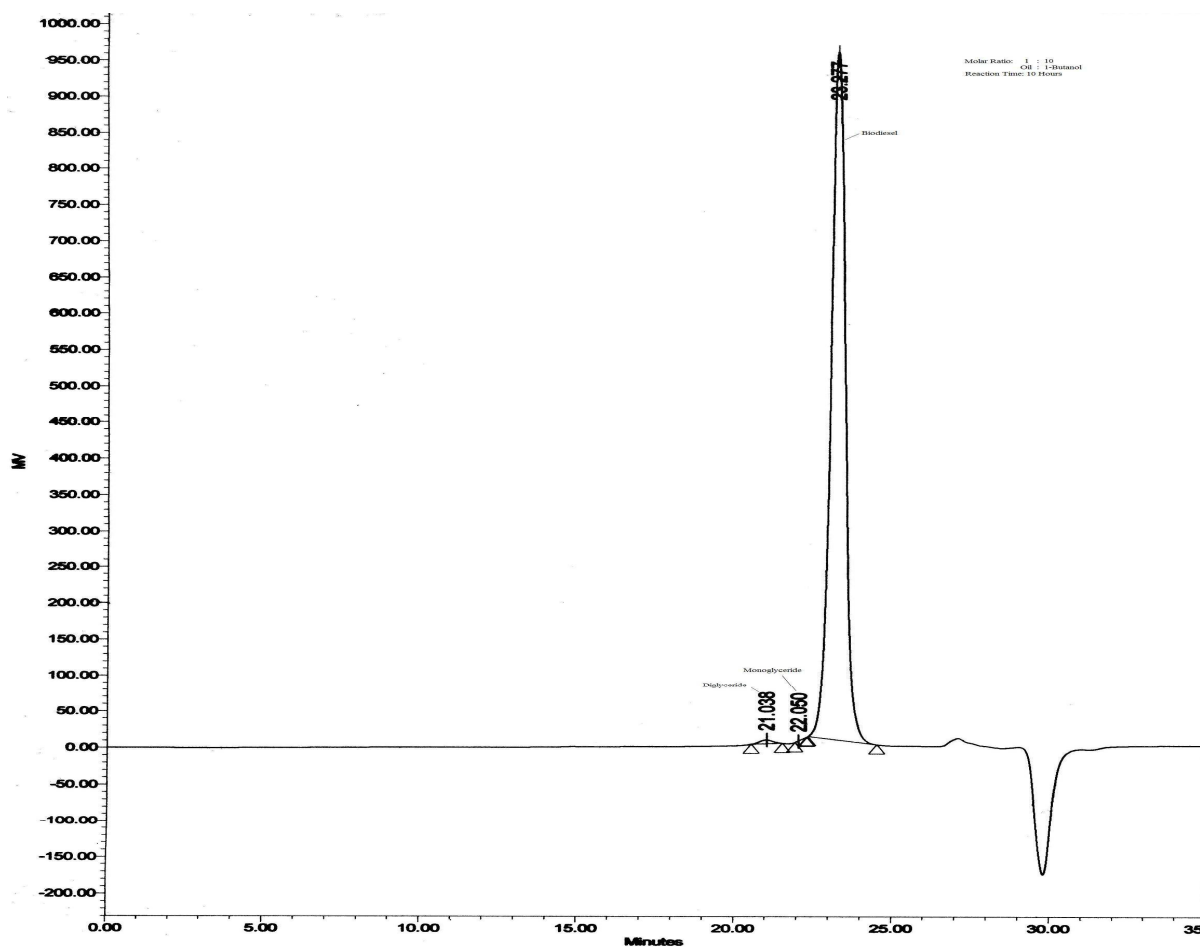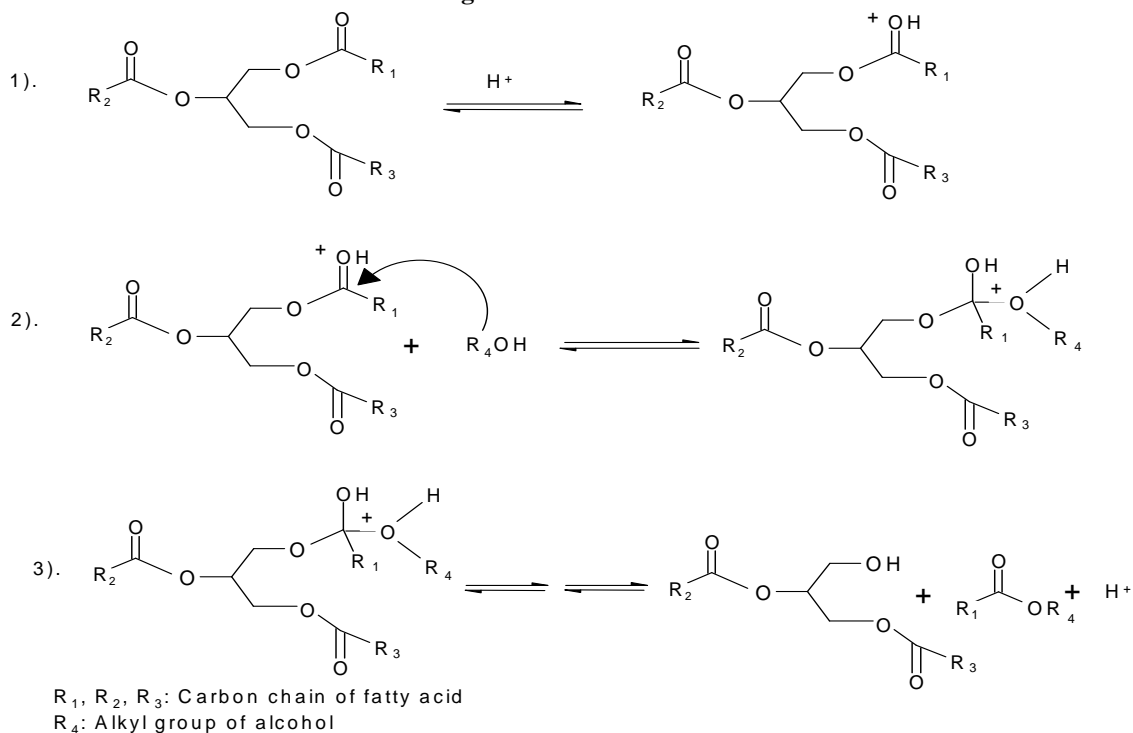
Scheme 3 Homogeneous acid-catalyzed reaction mechanism for the transesterification of triglycerides:
(1) protonation of the carbonyl group by the acid catalyst; (2) nucleophilic attack of the alcohol, forming a tetrahedral intermediate; (3) proton migration and breakdown of the intermediate. The sequence is repeated twice.

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RESULTS AND DISCUSSION

The Acid catalyzed transesterification process does not enjoy the same popularity as the base catalyzed process. The fact that the acid catalyzed reaction is about 4000 times slower than the base catalyzed reaction has been one of the main reason [23]. However, acid catalyzed transesterifications hold an important advantage with respect to base catalyzed ones: the performance of acid catalyst is not strongly affected by the presence of FFAs in the feedstock. In fact, acid catalyst can simultaneously catalyze both esterification and transesterification of FFA and TGs respectively to biodiesel. The acid catalyzed esterification of FFA follows a mechanistic scheme similar to tranesterification. Accordingly, instead of starting with a TG molecule, as in the transesterification reaction (Scheme 3), the starting molecule is FFA.

Thus, a great advantage with acid catalyst is that they can directly produce biodiesel from low cost feed stocks, generally associated with high FFA concentrations and thus, lowering the cost of production. As refining of crude oil costs around $ 0.12 per liter and were applied to the final cost of biodiesel production [24]. To achieve this CDGCSO with 5% FFA concentration was used as a feed stock and the maximum yield of biodiesel at optimum molar ration of oil:1-butanol were study. The results of percentage conversion of FFA and TGs to corresponding DG, MG and biodiesel are summarized in Table 2 to 6.

Canakci & Van Gerpen studied how the molar ratio affected reaction rates and product yield in the transmethylation of soybean oil by sulphuric acid [18]. And their results indicated that ester formation increased with increasing the molar ratio, reaching its height value, 98.4% at 1:30 molar ratio. Crabe et. al. also determined the effect of molar ratio within the range of 1:3 -1:23 and concluded that the height molar ratio required for complete transmethylation could be found between 1:35 and 1:45 by extrapolation [25].

In present study best results were achieved with the 1:10 oil:1-butanol molar ratio. The percentage conversion of oil to biodiesel was affected drastically by changing the oil:1-butanol molar ratio under the same conditions (Figure 5). Decreasing the oil:alcohol molar ratio from 1:40 to 1:10, reduce the time of reaction from 24 hours to 10 hours for more than 99 % conversion. As 5% FFA can get esterified to biodiesel, there is yield improvement by 5% using SPA catalyzed process from low cost feed stocks. By using SPA catalyzed process, there is no need for strict feed stock specifications, as there is no soap formation and problem associated with layer separation. In short cost effective biodiesel can be produced from low cost feed stock by using SPA catalyzed process with minimum separation cost and without soap formation with remarkable improvement in overall percentage of yield.

Table 1. Specification of low cost feed stock biodiesel.

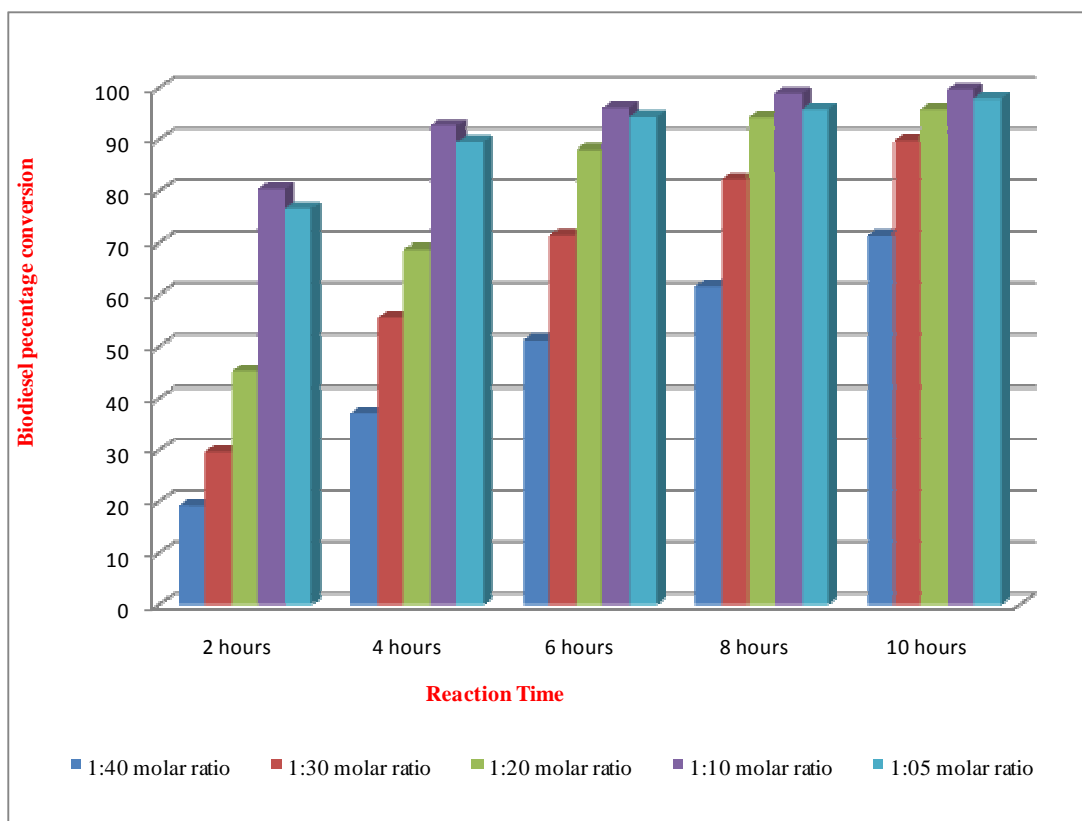| Sr. No.	Property	ASTM	Biodiesel	Units
1.	Free Glycerin	D6584	0.004	% mass
2.	Monoglyceride (MG)	D6584	0.179	% mass
3.	Diglyceride (DG)	D6584	0.160	% mass
4.	Triglyceride (TG)	D6584	0.181	% mass
5.	Total Glycerin	D6584	0.099	% mass
6.	Acid Number	D664	0.45	mg KOH/gm
7.	Water Sediment	D2709	0.045	% vol.
8.	Water by KF	D6308	0.075	ppm

Fig. 5 Effect of molar ratio on biodiesel percentage conversion with respect to reaction time.

Table 2. Results of percentage conversion of FFA and TGs to corresponding DG, MG and biodiesel (BD) with 1:40 oil:1-butanol molar ratio.

| Sr. No. | Feed Stock | Molar ratio (Oil: 1-butanol) | FFA (%) | Catalyst (SPA) (%) | Reaction Temp. (^{O}C) | Reaction Time (Hrs.) | % TG | % Conversion | | |
								% DG	% MG	% BD
1.	CDGCSO	1:40	5	5	120	2	61.42	18.50	0.89	19.19
2.	CDGCSO	1:40	5	5	120	4	38.14	24.08	0.74	37.04
3.	CDGCSO	1:40	5	5	120	6	24.85	23.34	0.63	51.18
4.	CDGCSO	1:40	5	5	120	8	16.43	21.50	0.51	61.56
5.	CDGCSO	1:40	5	5	120	10	10.50	17.69	0.46	71.35
6.	CDGCSO	1:40	5	5	120	12	6.76	14.32	0.32	78.60
7.	CDGCSO	1:40	5	5	120	14	4.26	11.05	0.27	84.42
8.	CDGCSO	1:40	5	5	120	16	2.29	7.15	0.21	90.35
9.	CDGCSO	1:40	5	5	120	18	1.31	4.62	0.15	93.92
10.	CDGCSO	1:40	5	5	120	20	0.72	3.22	0.09	95.97
11.	CDGCSO	1:40	5	5	120	22	0.5	2.30	0.07	97.83
12.	CDGCSO	1:40	5	5	120	24	0.10	0.37	0.00	99.53

Table 3. Results of percentage conversion of FFA and TGs to corresponding DG, MG and biodiesel (BD) with 1:30 oil:1-butanol molar ratio

Sr. No.	Feed Stock	Molar ratio (Oil: 1-butanol)	FFA (%)	Catalyst (SPA) (%)	Reaction Temp. (°C)	Reaction Time (Hrs.)	% Conversion			
							% TG	% DG	% MG	% BD
1.	CDGCSO	1:30	5	5	120	2	52.66	16.97	0.76	29.61
2.	CDGCSO	1:30	5	5	120	4	25.63	18.09	0.69	55.59
3.	CDGCSO	1:30	5	5	120	6	13.72	14.28	0.56	71.44
4.	CDGCSO	1:30	5	5	120	8	6.86	10.40	0.50	82.24
5.	CDGCSO	1:30	5	5	120	10	3.43	6.20	0.60	89.77
6.	CDGCSO	1:30	5	5	120	12	1.66	3.39	0.49	94.46
7.	CDGCSO	1:30	5	5	120	14	0.73	2.11	0.36	96.80
8.	CDGCSO	1:30	5	5	120	16	0.33	1.24	0.15	98.28
9.	CDGCSO	1:30	5	5	120	18	0.04	0.45	0.03	99.21
10.	CDGCSO	1:30	5	5	120	20	0.04	0.20	0.00	99.76

Table 4. Results of percentage conversion of FFA and TGs to corresponding DG, MG and biodiesel (BD) with 1:20 oil:1-butanol molar ratio.

Sr. No.	Feed Stock	Molar ratio (Oil: 1-butanol)	FFA (%)	Catalyst (SPA) (%)	Reaction Temp. (°C)	Reaction Time (Hrs.)	% Conversion			
							% TG	% DG	% MG	% BD
1.	CDGCSO	1:20	5	5	120	2	28.12	26.18	0.56	45.14
2.	CDGCSO	1:20	5	5	120	4	11.50	19.11	0.59	68.80
3.	CDGCSO	1:20	5	5	120	6	4.26	7.19	0.48	88.07
4.	CDGCSO	1:20	5	5	120	8	1.80	3.31	0.39	94.50
5.	CDGCSO	1:20	5	5	120	10	0.70	3.12	0.18	96.00
6.	CDGCSO	1:20	5	5	120	12	0.50	1.32	0.09	98.09
7.	CDGCSO	1:20	5	5	120	14	0.02	0.43	0.04	99.51
8.	CDGCSO	1:20	5	5	120	16	0.00	0.35	0.00	99.65

Table 5. Results of percentage conversion of FFA and TGs to corresponding DG, MG and biodiesel (BD) with 1:10 oil:1-butanol molar ratio.

Sr. No.	Feed Stock	Molar ratio (Oil: 1-butanol)	FFA (%)	Catalyst (SPA) (%)	Reaction Temp. (°C)	Reaction Time (Hrs.)	% Conversion			
							% TG	% DG	% MG	% BD
1.	CDGCSO	1:10	5	5	120	2	8.83	10.32	0.42	80.43
2.	CDGCSO	1:10	5	5	120	4	2.18	4.69	0.32	92.81
3.	CDGCSO	1:10	5	5	120	6	1.80	1.58	0.27	96.35
4.	CDGCSO	1:10	5	5	120	8	0.52	0.36	0.21	98.91
5.	CDGCSO	1:10	5	5	120	10	0.00	0.16	0.05	99.79

Table 6. Results of percentage conversion of FFA and TGs to corresponding DG, MG and biodiesel (BD) with 1:05 oil:1-butanol molar ratio.

Sr. No.	Feed Stock	Molar ratio (Oil: 1-butanol)	FFA (%)	Catalyst (SPA) (%)	Reaction Temp. (°C)	Reaction Time (Hrs.)	% Conversion			
							% TG	% DG	% MG	% BD
1.	CDGCSO	1:05	5	5	120	2	12.07	10.93	0.20	76.80
2.	CDGCSO	1:05	5	5	120	4	3.04	7.22	0.21	89.53
3.	CDGCSO	1:05	5	5	120	6	0.54	4.80	0.07	94.59
4.	CDGCSO	1:05	5	5	120	8	0.16	3.31	0.50	96.03
5.	CDGCSO	1:05	5	5	120	10	0.12	1.61	0.31	97.96
6.	CDGCSO	1:05	5	5	120	12	0.09	0.50	0.15	99.26

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CONCLUSION

In this study SPA catalyzed transesterification reaction of crude degummed cotton seed oil was investigated. As mention earlier, the oil:alcohol molar ration is an important parameter for transesterification reaction. From the obtained results, it can be evaluated that 1:10 oil:alcohol gives best results and reaction time decreases with decrease in molar ratio from 1:40 to 1:10. In present study 1:10 oil:1-butanol molar ratio, 5% SPA catalyst, 120^OC reaction temperature and 10 hours of stirring are considered to be the best condition to develop low cost method to produce biodiesel from crude degummed cotton seed oil.

As SPA catalyzed biodiesel production converts FFA and TG to biodiesel, there is no need to make TG free from FFA and it gets converted to biodiesel thus, increasing the percentage yield and ultimately reduce the cost for final product as there is no need to remove FFA from crude degummed cotton seed oil. Further there is decrease in molar ratio from 1:40 to 1:10 for height conversion at shortest time, there is also reduce cost for separation and recovery of alcohol that accounts for final cost of biodiesel. In short cost effective biodiesel can be produced by using SPA catalyzed process from low cost feed stock.

REFERENCES

[1] J. Van Gerpen, B. Shanks, R. Pruszko, D. Clements and G. Knothe, *Biodiesel production technology* NREL/SR-510-36244. Springfield, VA: Iowa State University and Renewable Products Laboratory USDA/NCAUR, **2004**.
[2] E. Lotero, Y. Liu, D. E. Lopez, K. Suwannakarn, D. A. Bruce and J. G. Goodwin Jr., *Ind. Eng. Chem. Res.,* Vol. 44 No. 14, **2005**, 5353-5363.
[3] L. G. Schumacher, S. C. Borgelt, D. Fosseen, W. Goetz and W. G. Hires, *Bioresour. Technol.,* Vol. 57 No. 1, **1996**, 31-36.
[4] J. A. Kinast and K. S. Tyson, Production of biodiesel from multiple feedstocks and properties of biodiesel and biodiesel/diesel blends, Final report; NREL: Golden, Colorado 80401-3393, 2003.
[5] A K. Singh, S. D. Fernando and R. Hernandez, *Energy Fuels,* Vol. 21, **2007**, 1161–1164.
[6] J. M. Marchetti, V. U. Miguel and A. F. Errazu, *Renew. Sust. Energ. Rev.,* Vol. 11, **2007**, 1300-1311.
[7] E. Santacesaria, R. Tesser, M. D. Serio, M. Guida, D. Gaetano, A. G. Agreda and F. Cammarota, *Ind. Eng. Chem. Res.,* Vol. 46, No. 25, **2007**, 8355-8362.
[8] R. Teall and R. F. Sickels, US Patent 6,979,426, **2005**.
[9] F. Ma and M. A. Hanna, *Bioresour. Technol.,* Vol. 70, No. 1, **1999**, 1–15.
[10] M. D. Serio, M. Ledda, M. Cozzolino, G. Minutillo, R. Tesser and E. *Ind. Eng. Chem. Res.,* Vol. 45, **2006**, 3009–3014.
[11] M. D. Serio, R. Tesser, M. Dimiccoli, F. Cammarota, M. Nastasi and E. *J. Mol. Catal. A,* Vol. 239, **2005**, 111-115.
[12] J. Van Gerpen, *Fuel Process Technol.,* Vol. 86, **2005**, 1097-1107.
[13] M. J. Haas, *Lipid Technol.,* Vol 16, **2004**, 7-11.
[14] P. Talley, *Biodiesel. Render*, **2004**.
[15] U. Schuchardta, R. Serchelia and R. M. Vargas, *J. Braz. Chem. Soc.,* Vol. 9, No. 1, **1998**, 199–210.
[16] K. S. Liu, J. *Am.Oil Chem Soc.,* Vol. 71, **1994**, 1179-1187.
[17] B. Freedman, E. H. Pryde and T. L. Mounts, *J. Am. Oil Chem.Soc.*, Vol. 61, No. 10, **1984**, 1638-1643.
[18] M. Canakci and J. Van Gerpen, Trans. *ASAE,* Vol. 42, **1999**, 1203-1210.

[19] S. Zheng, M. Kates, M. A. Dube and D. D. McLean, *Biomass Bioenergy,* Vol. 30, **2006**, 267-272.

[20] M. J. Goff, N. S. Bauer, S. Lopes, W. R. Sutterlin and G. J. Suppes, *J. Am. Oil Chem. Soc.,* Vol. 81, **2004**, 415-420.

[21] B. Freedman, R. O. Butterfield and E. H. Pryde, *J. Am. Oil Chem. Soc.,* Vol. 63, **1986**, 1375-1380.

[22] M. A. Dube, S. Zheng, D. D. McLean and M. A. Kates, *J. Am. Oil Chem. Soc*., Vol. 81, **2004**, 599-603.

A. Srivastava and R. Prasad, *Renewable Sustainable Energy Rev.,* Vol. 4, **2000**, 111-133.

[23] M. Kojima and T. Johnson Potential for Biofuels for Transport in Developing Countries, Energy *Sector Management Assistance Program,* E. S. M. A. Program, Joint UNDP / World Bank, **2005**.

[24] E. Crabbe, C. Nolasco-Hipolito, G. Kobayashi, K. Sonomoto and A. Ishizaki, *Process Biochem.,* Vol. 37, **2001**, 65-71.