

Biodiesel from Algae Oil with High Free Fatty Acid over Amphiphilic Solid Acid Catalyst

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Microalgae are an alternative source for biodiesel production, because they have more advantages than crop plants, i.e. high lipid content, can grow in freshwater or saltwater, faster growth rate and high productivity. In this context, our research was focused on the production of microalgae biomass from *Scenedesmus opoliensis* grown in mixotrophic conditions with glycerol as cheap carbon source and valorization of extracted algal oil as biodiesel. However, the extracted oil has high free fatty acids (FFA) and requires pre-treatment, if homogeneous catalysts are used due to saponification phenomenon and post-production processes. To avoid this inconvenience, the biodiesel was obtained in two steps: esterification of FFA with methanol over amphiphilic $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$ superacid catalyst and transesterification over KOH.

The amphiphilic superacid catalyst was prepared by surface modification of $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$ with n-octadecyltrichlorosilane and characterized using X-ray diffraction, TGA analysis, FT-IR spectroscopy and BET analysis. The FT-IR and TGA analysis confirm the attachment of alkylsilane on the surface of $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$ support.

The effect of various reaction parameters such as algal oil/alcohol molar ratio, catalyst amount and reaction time on catalytic performance was studied. The acid esterification was carried out to reduce algae oil acidity from 29 % to less than 1 %. After this step, the yield of biodiesel after transesterification with KOH was over 96 %. To improve biodiesel pour point property two glycerol derivatives previously synthesized were blended with algal biodiesel. The results indicated an improvement of tested properties.

1. Introduction

During the last years biodiesel has gained huge popularity because it can replace the need for fossil fuels and has an eco-friendly and renewable nature (Mandotra et al., 2014). Traditional raw materials for biodiesel production are edible vegetable oils, animal fats (Mata et al., 2013) and other residual products (Caetano et al., 2012). However, the use of edible oils to produce biodiesel is threatening the food supplies and biodiversity, causing social and environmental problems in different developed countries (Domenico et al., 2013). In order to overcome these problems, the attention of the researchers was focused on finding alternative sources for biodiesel synthesis such as microalgae. Microalgae have a number of advantages including higher photosynthetic efficiency and biomass production compared to other energy crops, faster growth rate (Sibi et al., 2014) and most importantly does not compete with crops for land because it can grow anywhere in ponds, photobioreactors and waste water (Mandotra et al., 2014). However, the current estimated cost of producing microalgae biodiesel is still too high (Cerón-García et al., 2013). To decrease the costs for biodiesel production it is essential to increase biomass production productivity of lipids and reintegrate the by-products formed during process (Kong et al., 2013). In this context, recent studies (Liang et al., 2009) indicate that mixotrophic growth, in which glycerol is used as cheap carbon source for algae cultivation, is one potential

mode for increasing the biomass and algal oil content and reintegrate biodiesel by-product - glycerol in process.

Biodiesel production from algae is generally done by extraction of algae oil from biomass using mixtures of organic solvents or directly extraction from wet algal slurry (Chen et al., 2012). During long-term storage, cellular lipids can be degraded to free fatty acid (Chen et al., 2012). In this context, algae oils extracted from microalgae biomass are improper for biodiesel synthesis when classical homogeneous base catalysts are used because the high content in free fatty acids (FFA) decrease the catalytic activity due to production of soap. One alternative to overcome this limitation is using a two-step process which involves esterification reaction of algae oil with high free fatty acid content with methanol in order to convert FFA in FAME, followed by transesterification of pre-treated algae oil over homogeneous base catalyst. The base-catalyzed in situ process is preferred over acid-catalyzed process due to lower requirement of reagents, milder reaction conditions and a substantially higher FAME yield (Dong et al., 2013).

In this study the main objective is to obtain biodiesel using algae oil extracted from *Scenedesmus opoliensis* biomass in two steps: esterification of free fatty acids with methanol over alkylsilylated $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$ and transesterification on potassium hydroxide. The effects of various reaction parameters such as algal oil/alcohol molar ratio, catalyst amount, water/n-octadecyltrichlorosilane ratio and reaction time on catalytic performance were studied.

2. Experimental

2.1 Algae cultivation and harvesting

The green microalga *Scenedesmus opoliensis* AICB 141 strain, used in this research was obtained from Institute of Biological Research Cluj-Napoca, thanks to Mr. Nicolae Dragos. *Scenedesmus opoliensis* was grown in a 3 L photobioreactor (BIOSTAT PBR 2S, Sartorius, Germania) with modified BBM medium, supplemented with NaHCO_3 and NaNO_3 . The cultures were grown using the following conditions: temperature: 28 °C, light intensity (irradiance): 120 $\mu\text{E}/\text{m}^2\text{s}$ for 7 days, feeding with synthetic gas mixture containing: 7 % CO_2 , 14 % O_2 and 79 % N_2 at a 20 mL / min debit. To use the glycerine obtained as by-product in biodiesel synthesis the algal biomass was grown in mixotrophic conditions where crude glycerin was used as carbon source (Andruleviciute et al., 2014). The microalgae biomass was harvested by centrifugation and the cells sediment obtained was lyophilized. The lyophilized biomass was stored at 4 °C for further analysis and processing. An optimized procedure, with chloroform-methanol-water was developed for total lipids extraction from microalgae. The algal oil contains 29 % free fatty acids and has the following fatty acid profile: 21 % C16:0, 5 % C18:0, 14 % C18:1, 16 % C18:2, 30 % C18:3, 4 % C18:4. To remove the chlorophyll, phospholipids and carotene the algal oil was filtrated on bleaching earth.

2.2 Preparation of alkylsilylated $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$

The alkylsilylated $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$ catalyst was prepared in three steps. In the first step $\text{TiO}_2\text{-ZrO}_2$ mixed oxide was obtained by precipitation of aqueous solutions of $\text{ZrOCl}_2\cdot 8\text{H}_2\text{O}$ and TiCl_4 with 25 % NH_4OH to reach pH = 9. The white precipitate was aged under stirring at 80 °C for 2 h, filtered, washed with distilled water to remove chloride ions and dried at 110 °C. In the second step the obtained mixed oxide was impregnated with 1M H_2SO_4 (7.5 mL / g of support) by sonication under vacuum for 10 min, dried at 110 °C and calcined at 500° C. In the last step the alkylsilylation process was carried out according to the method reported by Nur et al., (2012). In a typical experiment, the suspension $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$ containing 0.5 g water per 1.0 g of $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$ was immersed in 5 mL toluene containing 500 μmol of n-octadecyltrichlorosilane and stirred for 5 min at room temperature. Then, the solid was collected and dried at 110 °C for 5 h. The sample was labeled partial- $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$. The fully modified $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$ was prepared without the addition of water and labeled as full- $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$.

2.3 Catalyst characterization

The samples were characterized by X-ray diffraction performed with a Bruker D8 Advance diffractometer with $\text{CuK}\alpha$ radiation ($\lambda_{\text{Cu}} = 1.5406 \text{ \AA}$). Data were recorded from 10° to 80° with steps of 0.01 deg.min⁻¹. The BET analyses were measured by on a Quantachrome ChemBet-3000 instrument at liquid nitrogen temperature using degassed samples. The FT-IR spectra were recorded with a Tensor 27 FT-IR Spectrometer Bruker, using KBr disks. Thermogravimetric analyses of the compounds were measured with a TGA/SDTA 851Mettler Toledo in the temperature range 20-700 °C. The total acidity of the solid samples was measured by thermogravimetric analysis of adsorbed n-butylamine (Pedrosa et al., 2006).

2.4 Esterification reaction

The catalytic activity of the three prepared catalysts was tested in esterification reaction of free fatty acids from extracted algae oil with methanol at atmospheric pressure. The reactions were carried out using a 50 mL Erlenmeyer flask equipped with a reflux condenser, magnetic stirrer, heated with a magnetic stirrer plate. The experiments were performed at methanol temperature reflux. The flask was filled with algae oil, catalyst and methanol and heated under constant agitation speed of 600 rpm. In order to decrease acidity value under 1% the following parameters were varied: algal oil/alcohol molar ratio, catalyst amount and reaction time. After completion of the reaction, the mixture was filtrate to remove catalyst and the excess of methanol was recovered by rotary evaporator. The acid value was determined by titration method (Standard STAS 145-67) and expressed as oleic acid (Acidity (%) = $282 \cdot v \cdot n / 1000m \cdot 100$; where v = volume (mL) of KOH solution; n = normality, m = sample weight).

2.6 Transesterification reaction

The pre-treated oil obtained from esterification reaction was subjected to transesterification over potassium hydroxide using the method outlined by Stepan et al., (2008). The biodiesel product was analyzed using GC-MS/MS TRIPLE QUAD (Agilent 7890 A) equipped with a DB-WAX capillary column (30 m length, 0.25 mm internal diameter, 0.25 μ m film thickness). The oven temperature was initially set at 70 °C increasing to 230 °C with 4 °C /min and hold time 5 min. The GC injector and MS ion source temperatures were 250 °C and 150 °C, respectively. The fatty acid methyl ester peaks in the biodiesel sample were identified using NIST MS database. The quantitative determination was done by using methyl-heptadecanoate as an internal standard. Pour point properties of biodiesel and biodiesel blends with two glycerol derivatives were determined according to the EN ISO 3016:1994 standard.

3. Results and Discussion

3.1 Catalyst characterization

XRD patterns recorded for the three catalysts are presented in Figure 1. As can be seen the alkylsilylation process not influence the crystalline structure of $\text{SO}_4^{2-} / \text{TiO}_2\text{-ZrO}_2$. For this reason only the $\text{SO}_4^{2-} / \text{TiO}_2\text{-ZrO}_2$ was discussed. $\text{SO}_4^{2-} / \text{TiO}_2\text{-ZrO}_2$ exhibit anatase as crystallization phase ($2\theta = 25^\circ, 38^\circ, 48^\circ$ and $54^\circ, 66^\circ$) with tetragonal geometry (Aisiyiah et al., 2010). Furthermore, no characteristic peaks were observed for ZrO_2 , indicating that zirconium ions reacted strongly with the titanium ions (Zhao, et al., 2009). To support this affirmation unit cell volume was calculated for $\text{SO}_4^{2-} / \text{TiO}_2$ and $\text{SO}_4^{2-} / \text{TiO}_2\text{-ZrO}_2$. The results indicate an increase in unit cell volume from 132.60 Å for $\text{SO}_4^{2-} / \text{TiO}_2$ to 135.35 Å for $\text{SO}_4^{2-} / \text{TiO}_2\text{-ZrO}_2$, due to the incorporation in the titanium network of zirconium ions which have a larger ionic radius (0,800 Å) compared to titanium (0.605 Å). Using Debye–Scherrer equation (for the most intense peak at $2\theta = 25^\circ$) the crystallite size was calculated. The value obtained (22.45 nm) indicates a good degree of crystallization.

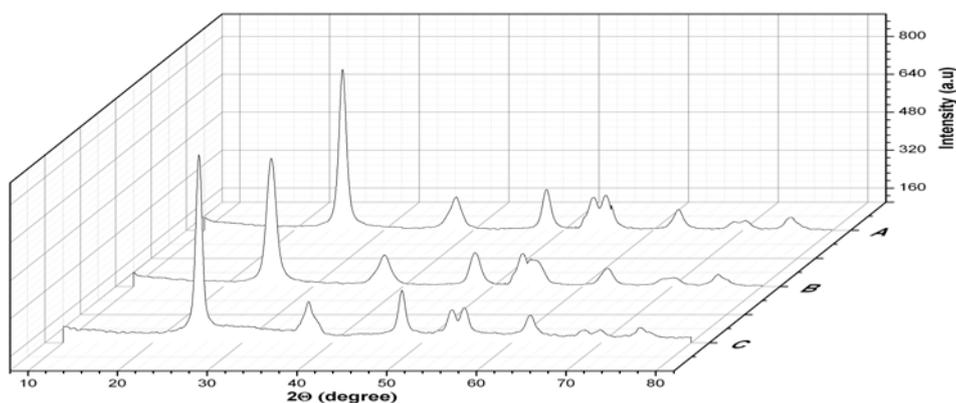


Figure 1: X-ray diffraction patterns of full- $\text{SO}_4^{2-} / \text{TiO}_2\text{-ZrO}_2$ (A), partial- $\text{SO}_4^{2-} / \text{TiO}_2\text{-ZrO}_2$ (B) and $\text{SO}_4^{2-} / \text{TiO}_2\text{-ZrO}_2$ (C)

The BET specific surface areas of $\text{SO}_4^{2-} / \text{TiO}_2\text{-ZrO}_2$, partial- $\text{SO}_4^{2-} / \text{TiO}_2\text{-ZrO}_2$ and full- $\text{SO}_4^{2-} / \text{TiO}_2\text{-ZrO}_2$ were 85.52, 84.38 and 84.12 m^2/g respectively. The obtained values for surface areas of the functionalized and untreated $\text{SO}_4^{2-} / \text{TiO}_2\text{-ZrO}_2$ are similar, which suggest that the surface modification of $\text{SO}_4^{2-} / \text{TiO}_2\text{-ZrO}_2$ does not influence the specific surface area. The results are consistent with literature data (Zapada et al., 2012).

The FT-IR spectra of the three catalysts are presented in Figure 2. As can be seen all spectra presents a broad absorption peak around 3300 cm^{-1} and a sharp one around 1620 cm^{-1} associated to vibration of water molecules adsorbed on the catalysts surface. The attachment of the alkyl chains on the $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$ surface is confirm by the two anti-symmetric and symmetric C–H stretching bands of methylene group recorded at 2919 and 2850 cm^{-1} . These results are consistent with observation made by (Jing et al., 2011). The characteristic bands of vibrational modes of sulfate groups impregnated on $\text{TiO}_2\text{-ZrO}_2$ support are observed in the region $1250\text{-}990\text{ cm}^{-1}$.

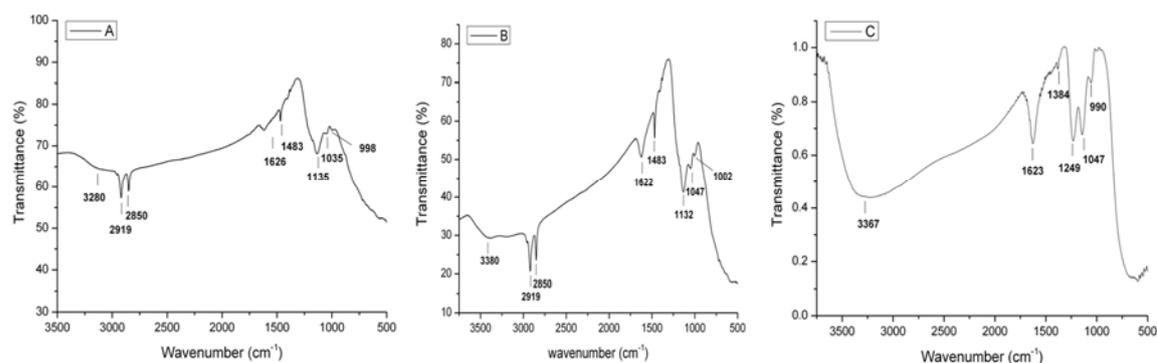


Figure 2: FT-IR spectra of full- $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$ (A), partial- $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$ (B) and $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$ (C)

Thermogravimetric analysis was used to measure the synthesized catalysts and to test stability of n-octadecyltrichlorosilane coat on $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$ surface. The thermogravimetric analysis for partial- $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$ and respectively full- $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$ catalyst exhibited 2 weight losses. The first weight loss in the temperature range $20\text{-}350\text{ }^\circ\text{C}$ is caused by the loss of surface and interlayer water; while the second mass loss around $450\text{-}500\text{ }^\circ\text{C}$ is attributed to decomposition of the silane. Song and Sand (2001) reported the same behavior for organic coatings on the clays. The same trend was observed for $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$ catalyst with the mention that the second lost occurred in the range $550\text{-}650\text{ }^\circ\text{C}$ and corresponds to the decomposition of sulfate species impregnated on $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$ surface.

The surface acidity measurements for the three catalysts indicate a total acidity of 1.03, 1.06 and 1.05 millimoles n-butylamine/g catalyst for $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$, partial- $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$ and respectively full- $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$, which suggest that alkylsilylation process does not influence the acidity property of $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$ catalyst.

3.2 Esterification reaction

To determine the optimal parameters $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$ was chosen as catalyst for esterification reaction of free fatty acids with methanol. The influence of various reaction parameters such as algal oil/methanol molar ratio, catalyst amount and reaction time on catalytic performance of was studied (Table 1).

The first parameter studied was algal oil/methanol molar ratio. The molar ratios of oil to methanol tested were 1:3, 1:6, 1:9 and 1:12. Experimental data presented in Table 1 indicates that when methanol concentration increases, acidity decrease to 1.57 % at a molar ratio oil to methanol of 1:9. Probably because the equilibrium was reached, after this value the conversion of FFA slightly changed. The excess of methanol can be recovered and reused in esterification reaction of FFA. The effect of catalyst amount on algae oil acidity was tested using different amounts from 2 to 8 wt. % based on algae oil. The results show that, when the catalyst dosage is increased from 2 wt. % to 6 wt. %, the acidity decreases from 29 % to 1.57 %. But with further increase in amount of catalyst no improvement in FFA conversion was observed. This behavior can be explained by the increasing number of acid centers until reaches a maximum after which due to the pores blocking by reactants the reaction stagnates. The influence of reaction time on algae oil acidity was also investigated. The evolution in time shows a continuous growing trend. At the beginning of the reaction, the reaction rate is high, after which the conversion rate slows down. After 5 h the acidity value was 1.57 %.

Previously determined optimal parameters (molar ratios of oil to methanol 1:9, catalyst amount 6 wt. % based on algae oil and 5 h reaction time) were applied to esterification reaction of FFA with methanol over partial- $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$ and full- $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$. Experimental data indicates that using amphiphilic catalysts obtained by partial or full impregnation of $\text{SO}_4^{2-}/\text{TiO}_2\text{-ZrO}_2$ surface with n-octadecyltrichlorosilane, the acidity

decrease from 1.57 % to 0.89 % and respectively 0.91 % for partial-SO₄²⁻/TiO₂-ZrO₂ and respectively full-SO₄²⁻/TiO₂-ZrO₂ and the reaction time decrease to 4 h, due to fact that alkylsilylated SO₄²⁻/TiO₂-ZrO₂ is placed at the interface of the hydrophobic triglyceride molecules and hydrophilic alcohol.

Table 1: Parameters optimization of esterification reaction over SO₄²⁻ / TiO₂-ZrO₂

Time reaction (h)	Catalyst loading (wt.% algae oil)	Molar ratio algae oil : methanol	Acidity
5	6	1:3	4.98
5	6	1:6	2.23
5	6	1:9	1.57
5	6	1:12	1.60
5	2	1:9	5.40
5	4	1:9	3.34
5	6	1:9	1.57
5	8	1:9	1.61
1	6	1:9	3.78
2	6	1:9	2.55
3	6	1:9	2.09
4	6	1:9	1.89
5	6	1:9	1.57
6	6	1:9	1.58

The stability of SO₄²⁻/TiO₂-ZrO₂, partial-SO₄²⁻/TiO₂-ZrO₂ and full-SO₄²⁻/TiO₂-ZrO₂ was tested by recycling them five runs. After each experiment, the catalysts were filtered and dried 6 h at 110°C. The reused SO₄²⁻/TiO₂-ZrO₂ catalyst show a decrease trend of acidity value with 25 %, probably due to sulfate leaching. Instead, regarding the amphiphilic catalysts a negligible decrease in catalytic activity was recorded for the two amphiphilic solid acid catalyst, indicating that by coating SO₄²⁻/TiO₂-ZrO₂ surface with n-octadecyltrichlorosilane the loss of sulfate anions that are responsible for the formation of acidic centers is prevented.

The pre-treated algae oil having an acidity of 0.89 % was subjected to transesterification reaction using an alkali catalyst. The transesterification reaction was carried out using the method described by Stepan et al., (2008). The yield obtained in biodiesel from algae oil was 96.45%.

The cold flow property is an important performance indicator for diesel fuels and biofuels because it affects diesel engine fuel supply at low temperatures (Pegmei et al., 2013). Unfortunately biodiesel is susceptible to have start-up and performance problems when vehicles and fuel systems are subjected to cold temperatures (Perez et al., 2010). To improve pour point property of algae biodiesel two glycerol derivatives ((2-ethyl-2-methyl-1,3-dioxolan-4-yl)methanol and (2-ethyl-2-methyl-1,3-dioxolan-4-yl)methyl hexanoate) synthesized into a previous study using the same class of catalyst (Oprescu et al., 2014) were blended in a ratio of 3 wt. % with algal biodiesel. The results indicated for the two blends an improvement of pour point properties from - 5 °C to -7 and -11°C respectively, for 2-ethyl-2-methyl-1,3-dioxolan-4-yl)methyl hexanoate and (2-ethyl-2-methyl-1,3-dioxolan-4-yl)methyl hexanoate respectively.

4. Conclusion

This study presents algae biodiesel production in two-steps. Esterification of free fatty acids was realised using three catalysts SO₄²⁻/TiO₂-ZrO₂, partial- SO₄²⁻/TiO₂-ZrO₂ and full-SO₄²⁻/TiO₂-ZrO₂. The acidity of algae oil was decreased from 29 % to less than 1 %, with optimized reaction parameters. The experimental data obtained shows that by modification of SO₄²⁻ / TiO₂-ZrO₂ surface with n-octadecyltrichlorosilane the catalytic activity increase. The high activity of the two amphiphilic catalysts is explained by the fact that by coating SO₄²⁻/TiO₂-ZrO₂ surface with n-octadecyltrichlorosilane the loss of sulfate anions is prevented and the catalysts are placed at the interface of the hydrophobic and hydrophilic phases. After this step, the yield obtained in biodiesel from algae oil was 96.45 %. To improve biodiesel pour point property two glycerol derivatives were blended with algal biodiesel in a ratio of 3 wt. %. The results indicate for the two biodiesel blends a decrease pour point temperature up to 6 °C

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