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and Mochamad Zakki Fahmi*

APPLICATION OF GLUTARALDEHYDE-CROSSLINKED CHITOSAN MEMBRANES FROM SHRIMP SHELLWASTE ON PRODUCTION OF BIODIESEL FROM *CALOPHYLLUM INOPHYLLUM* OIL

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Abstract. The purification of biodiesel was performed using glutaraldehyde-crosslinked chitosan membrane to improve the quality and to reduce the cost of biodiesel production. Biodiesel was obtained by acid-based catalyzed transesterification of *Calophyllum inophyllum* oil. The results of this experiment showed that the amount of glycerol in biodiesel before the process is 0.3014 ± 0.05 %. After the purification process performed for 1 h, the glycerol content in biodiesel has been reduced to 0.1429 ± 0.09 %.

Keywords: biodiesel, glycerol, crosslink, chitosan, membrane.

1. Introduction

Since last two decades the prediction on increasing demand of petroleum has been established, it was coupled with limitation on accessible oil [1]. The availability of oil feed stocks is a main reason in a push to more sustainable alternatives. Among sustainable supplies, renewable resources that perform short cycle, were fully considered. Covering these problems, biodiesel promises to be an alternative for fossil fuels as a source of carbon for the chemical and material needs. Biodiesel is an attractive substitute for petro-diesel fuel because it is environmentally beneficial, thus has a biodegradable and low gas emission profile [2]. Biodiesel usually in the form of fatty acid methyl esters (FAME) is often derived from either the transesterification of triglycerides or the esterification of free fatty acids (FFA) with methanol. As a substitute to diesel fuel, biodiesel is commonly produced

from renewable natural sources such as vegetable oils, animal fats and microalga oil. Biodiesel is considered to be carbon neutral because biodiesel yielding plants such as *Jatropha curcas*, rape plant and palm trees absorb carbon-dioxide to a greater extent than that used as a fuel in diesel engines.

During biodiesel synthesis, glycerol is the main by-product which has different phases from biodiesel. So far, glycerol is widely used for pharmaceuticals, cosmetics, foods, polymers, surfactants and lubricants [3]. However, the presence of glycerol in biodiesel is undesirable because it is responsible for fuel tank damage, decreasing engine performance [4] and acrolein releasing into the atmosphere [5], which takes place when fuel burning occurs at the temperatures above 453 K. Therefore, the amount of glycerol in the fuel should be reduced to a minimum and it makes biodiesel development as a major issue regarding strategies on its developing on commercial scale [6]. Membrane technology has been applied in various industrial processes such as petrochemical, vegetable oil processing, food and beverage industries, desalination of sea water, pharmaceuticals, sterilization of bacteria, industrial wastewater treatment, purification of VCO, sugar cane extract refining, etc. [7]. Introducing membrane for biodiesel purification was proposed by Dube *et al.* [8] who built a membrane carbon reactor in a biodiesel reactor. The membrane successfully separated unreacted oils, thus generating a high purity biodiesel and shifting the equilibrium towards products. Wang *et al.* [9] further used ceramic membrane for biodiesel purification, where membrane good properties were shown, including solvent resistance, high temperature resistance, high

mechanical strength and easy making. However, ceramic has disadvantages like brittleness, expensive production cost due to energy consumption and needing of high temperature for its production [2]. Therefore, improvement of research in developing other membranes from suitable materials, like natural polymers, was a critical issue to overcome limitations of membrane application relating to creation of high quality membrane for biodiesel production with mechanical properties and high thermal stability at low production cost. However, the distinctive features performed by the natural polymer, such as biodegradability, sustainability, super-hydrophobicity and high surface area, became the main consideration on application natural polymer at water-oil separation [10].

Among several natural polymers explored, chitosan was a common choice for many applications in medicine [11], food [12] and industry processing [13]. This material that can be created from skin of crustaceans performs excellent properties relating to biocompatibility, nontoxicity, and biodegradability [14]. However, application of crosslinked chitosan as a membrane for biodiesel purification was not deeply explored so far.

The present work was aimed to find alternative methods for biodiesel purification using the membrane to increase biodiesel production efficiency and reduce environmental pollution. Utilized biodiesel was produced from transesterification of *Calophyllum inophyllum* (CI) oil. Moreover, the obtained chitosan membrane was further crosslinked with glutaraldehyde to improve its mechanical properties. Several tests and measurements were carried out to determine the membrane mechanical properties and its performance (flux and rejection).

2. Experimental

2.1. Materials

In this research we used pro analysis grade chemicals, including HCl, NaOH, acetic acid, methyl heptadecanoate, phenolphthalein indicator, acetone, Na₂CO₃ (purchased from Sigma Aldrich, USA), *n*-hexane, Na₂SO₄ anhydrous, NaIO₄ and bromotymol blue. H₂SO₄, ethanol, KOH, glutaraldehyde, methanol were purchased from Merck, Germany. Shrimp shell was got from seafood market at Surabaya. CI oil was obtained by grinding of CI seed from Surabaya.

2.2. Experimental Procedure

2.2.1. Preparation of chitosan

Chitosan was created after several processes, namely deproteinization, demineralization, depigmen-

tation and deacetylation. For the first step we removed the protein found in the shell of the shrimp (deproteinization) by adding 3.5 % NaOH solution to the leather dust shrimp with the ratio between the shrimp shell and NaOH equal to 1:10 (mg/ml). Then we removed mineral from the shrimp shell (demineralization) by adding 2N HCl to the crude chitin obtained from the deproteinization process with crude chitin:HCl ratio of 1:15 (mg/ml). Depigmentation process was done by soaking the chitin in the acetone solvent for 30 min. Lastly we performed deacetylation to transform chitin into chitosan by adding 50 % NaOH solution into the chitin with chitin:NaOH ratio being 1:10 (mg/ml). The mixture was boiled at 368 K for 2 h.

2.2.2. Preparation of glutaraldehyde-crosslinked chitosan

For preparing the crosslinked membrane we first made dope by dissolving chitosan in 2 % acetic acid. We also varied weight ratio of glutaraldehyde to chitosan (2.5, 2.7, 2.9, 3.1 and 3.3 %). The dope was created by placing 100 mg of chitosan and 2.5 ml of 2 % acetic acid in a closed Erlenmeyer flask and stirring at room temperature using a magnetic stirrer until chitosan was completely dissolved and degassed overnight to eliminate air bubbles. The solution was further proceeded to form the membrane by the phase inversion method. After being washed with DI-water and dried, the membrane was soaked with glutaraldehyde for 1 h. The glutaraldehyde concentration was also varied within 0.01–0.05 %. The prepared membrane was characterized by determining the flux, rejection, morphology and mechanical properties with SEM. The flux (permeability) was calculated according to Eq. (1)

$$J = \frac{V}{A \cdot t} \quad (1)$$

where J – flux value, l·m⁻²·day⁻¹; t – time, days; V – volume of permeate, l; A – surface area of the membrane, m².

Rejection was determined by measuring biodiesel turbidity before and after passing the membrane, then we calculated the rejection by Eq. (2):

$$R = \left(1 - \frac{C_p}{C_f} \right) \cdot 100 \% \quad (2)$$

where R is the rejection coefficient; C_p is the concentration of a substance dissolved in permeate; C_f is the concentration of dissolved substances in feeds.

2.2.3. Characterization of CI oil

Before CI oil being proceeded to become biodiesel, we need to measure its FFA levels, water and fatty acid content and molecular weight. We measured acid value by dissolving 10 g of CI in a mixture of 95 % ethanol and

diethyl ether with ratio the 1:1 (v/v). Then we titrated the mixture with 0.1 N KOH. The acid value was determined by Eq. (3).

$$\text{Acid value} = \frac{V \cdot N \cdot 56.1}{m} \quad (3)$$

where m – the weight of CI; V and N – the volume and normality of KOH, respectively [15].

We measured water content of CI oil by heating ± 2.5 g of oil at 373 K for 1 h and placed it into a desiccator for 5 min before it was weighed. The water content in oil is equal to water that evaporates (Indonesian National Standard SNI 01-5009.12 -2001) and is measured using below equation:

$$\text{Water content} = \frac{W_1 - W_2}{W_1 - W} \cdot 100 \% \quad (4)$$

where W – the weight of the empty cup; W_1 – the weight of the cup and oil before heating; W_2 – the weight of the cup and oil after heating.

Molecular weight and fatty acid content were observed by GC-MS analysis.

2.2.4. Synthesis of biodiesel

For the synthesis of biodiesel from CI oil we used acid-based catalyzed transesterification following Berchmans and Hirata's report with some modification [16]. Briefly, CI oil was firstly treated by acid esterification to remove FFA from CI oil, where 0.3 mol of methanol was mixed with H_2SO_4 (1 % from weight of CI oil) and stirred after addition of 0.01 mol of CI oil at 333 K for 3 h. The resulted organic phase was further separated by centrifugation. The next process is transesterification reaction. It is the addition of resulted oil into the mix of methanol (0.3 mol) and NaOH with NaOH:oil ratio of 2.5 [16]. The process was maintained at 333 K for 3 h. The oil phase was then collected for purification process.

2.2.5. Purification process

Biodiesel obtained was further purified using glutaraldehyde-crosslinked chitosan membrane. Conversion rate of biodiesel can be determined by Eq. (5):

$$\text{Biodiesel conversion} = \frac{\sum A - A_s}{A_s} \cdot \frac{C_s V_s}{m} \cdot 100 \% \quad (5)$$

where $\sum A$ – total methyl ester peak areas; A_s – peak area of the internal standard solution (methyl heptadecanoate); C_s – concentration of STD solution (10 mg/ml); V_s – volume of STD solution (5 ml); m – amount of samples (250 mg). The amount of glycerol in biodiesel is determined according Indonesian National Standard (SNI 06-1564-1989).

$$\text{Glycerol}(100\%) = \frac{(T_1 - T_2) \cdot N \cdot 9.209}{W} \quad (6)$$

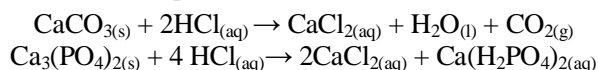
where T_1 – NaOH volume; T_2 , N – NaOH volume in titrated sample and blank test, respectively; W – weight of NaOH. Characterization of biodiesel quality was performed according to the standard ASTM D6751 and RSNI EB 020551, which include the determination of the levels of alkyl ester and glycerol, viscosity, flash point and cloud point.

2.2.6. Other characterizations

For characterization we used several instruments, including GC-MS, UV-Vis spectrophotometer, pH meter Scott, Differential Scanning Calorimetry (DSC), Autograph, Scanning Electron Microscopy (SEM), Fourier Transform Infrared (FTIR), Oswald Viscometer.

3. Results and Discussion

Chitosan was prepared by isolating chitin from the shrimp shell. Deproteinization process produces crude chitin or rough chitin and changes the shrimp shell color from reddish-brown to brownish-yellow; this is due to the termination of the chemical bond between chitin and proteins so that the pigment of the shrimp shell is dissolved. Crude chitin yield was 48.08 %. Demineralization is a process to remove inorganic compounds or minerals in the form of CaCO_3 and $\text{Ca}_3(\text{PO}_4)_2$ from the shrimp shells [17]. Minerals were removed by soaking crude chitin in 2N HCl solution, with the shrimp shell:HCl ratio being 1:15 (mg/ml), for 30 min at room temperature. The reaction that occurs during demineralization process is as follows:



The crude chitin gained from demineralization was 15.59 %. In the next process crude chitin was performed by soaking crude chitin in acetone solvents for 30 min for depigmentation. The obtained chitin yield was 14.22 %. The transformation of chitin to chitosan (deacetylation) was done by breaking the acetyl group bonds of chitin with nitrogen, so that it turned into an amine group (NH_2). This reaction produces white chitin with the yield of 24.2 %. Several characterizations, including a solubility test and a functional group analysis were conducted to determine the quality of chitin and chitosan obtained from isolation procedure. The solubility tests were done to determine the formation of chitosan. If the sample is soluble in 2 % acetic acid, the sample was chitosan, if insoluble – chitin. We further investigated the functional groups and determined the degree of deacetylation of chitin and chitosan using FTIR (Fig. 1). In general, FTIR

spectrum of chitosan in this study was quite similar with that of commercial chitosan and chitin performed in reported studies, where common vibrations at 3448 (i), 2931 (ii), and 1658 cm^{-1} (iii) corresponding to O–H, C–H, and C=C in the main chain, respectively, as well as at 1627 cm^{-1} (iv) for N–H vibration were observed [18]. Furthermore, the transformation of chitosan from chitin is confirmed by decreasing transmittance of C–N stretching of chitosan's amide group at 1319 cm^{-1} (v). Following the method described in the previous report [17], deacetylation degrees (%DD) of chitin and chitosan obtained in this research were 66.67 and 75.71 %, respectively. The average molar weight of chitosan determined by viscometric method was 552.6 kg/mol. Based on the literature, this value is in accordance with molar weight of normal chitosan, which average molar weight is within 190–700 kg/mol [19].

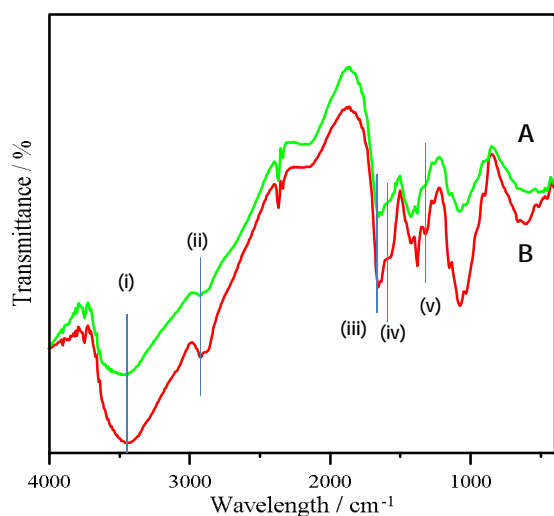


Fig. 1. FTIR spectra of prepared chitin (A) and chitosan (B)

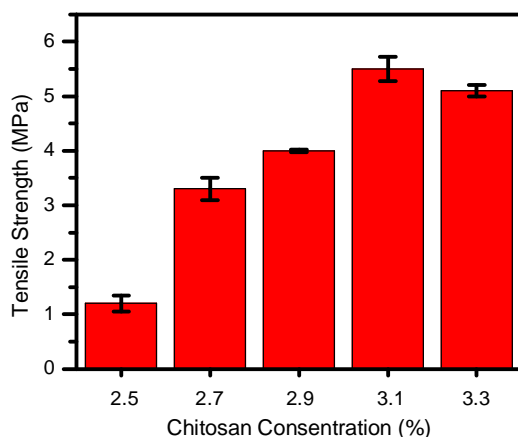


Fig. 2. Mechanical properties of chitosan varied membrane

Glutaraldehyde-crosslinked chitosan membrane was produced using the phase inversion method by evaporating the solvent after printing the membrane on the glass plate. A phase inversion method is based on the principle of thermodynamics, where the initial solution is stable. The inversion process emerges from instability conditions that change process from liquid to solid [20]. By varying chitosan concentration, the mechanical properties of the membranes were investigated (Fig. 2). Fig. 2 shows the correlation between chitosan concentration and its mechanical properties (tensile strength), where the tensile strength value of the membrane increases with the increase in chitosan concentration. Enhancement of chitosan content increases a number of physical attractions between molecules constructing membrane, such as hydrogen and van der Waals bonding. This condition is a responsible reason for this increasing stress value. However, the stress decreases when chitosan concentration is 3.3 %. The nature of chitosan, which is water-insoluble, interferes the membrane manufacturing and decreases the stress value. The highest stress value of the membrane (5.45 MPa) was reached at chitosan concentration of 3.1 %. Therefore, in the further process of manufacturing glutaraldehyde-crosslinked chitosan we used 3.1 % chitosan concentration. The crosslinking process was carried out with following glutaraldehyde concentrations: 0.01, 0.02, 0.03, 0.04 and 0.05 %. Tensile test of the membrane with the varied glutaraldehyde concentrations shows the effect of glutaraldehyde concentrations on the membrane mechanical properties (Fig. 3). The highest membrane stress is 6.6 MPa when glutaraldehyde concentration is about 0.05 %. Moreover, the greater the concentration of glutaraldehyde, the higher value of the stress. Crosslink bonding performed by glutaraldehyde and chitosan will improve the membrane mechanical properties.

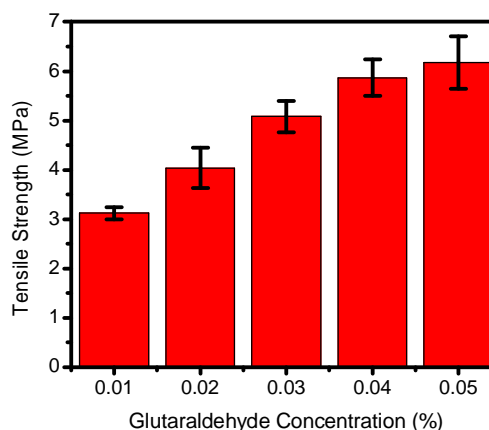


Fig.3. Mechanical properties of glutaraldehyde varied membrane

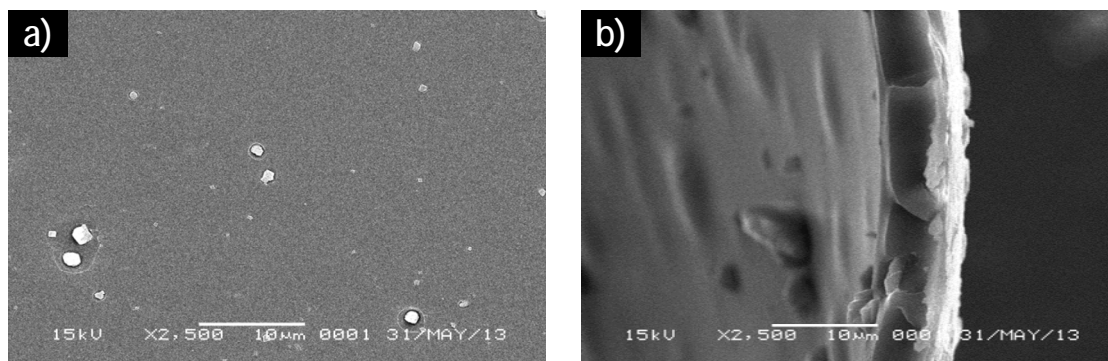


Fig. 4. SEM images of chitosan membrane before crosslinking: surface (a) and the transverse cross-section (b)

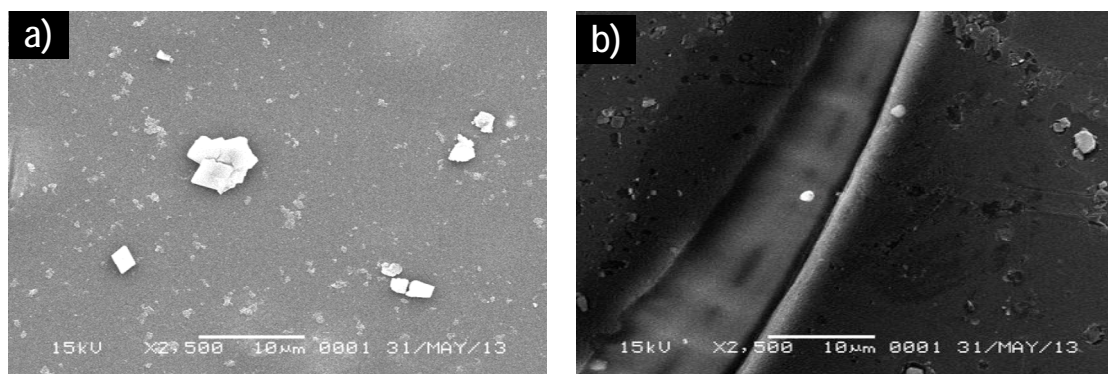


Fig. 5. SEM images of chitosan membrane after crosslinking process: surface (a) and the transverse cross-section (b)

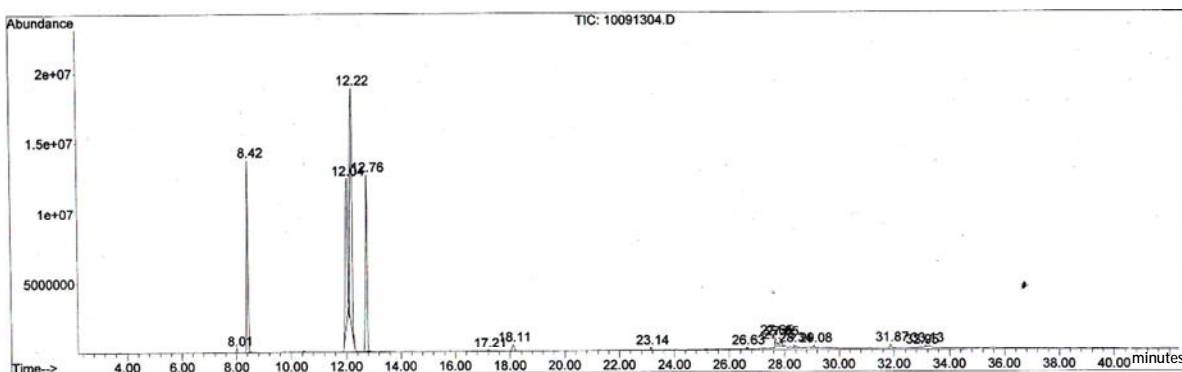


Fig. 6. GC-MS chromatogram of biodiesel from CI oil

Further morphology analysis of the membrane was observed by SEM to get images of membrane cross-section, pores size and distribution. The images of chitosan membrane cross-section and pores distribution with and without crosslinking agents are shown in Figs. 4 and 5. The images show that the membrane surfaces have plenty and uniform pores distribution with average pore size below 10 nm, hence the membranes are suitable for oil purification.

From the biodiesel analyses, moisture content of CI oil and FFA levels were 0.75 and 13.89 %, respectively. Because of having high FFA value (>2.5 %), the synthesis of biodiesel in this study uses two stages process to prevent

saponification, namely the stage of esterification and transesterification. Esterification process was performed to reduce the FFA levels (at ± 1 %). This process must be undertaken to prevent the saponification reaction between free fatty acids with alkaline catalyst in the transesterification process. It is noteworthy, that during biodiesel synthesis, soap can lead to the formation of an emulsion that can complicate the purification process. Biodiesel produced was subsequently analyzed by GC-MS to determine the type and content of its compounds (Fig. 6).

Chromatographic results show that biodiesel obtained from CI oil contains four major compounds. The compounds performing extensive areas at 18.367, 20.298,

35.432 and 20.036 % correspond to methyl palmitate, methyl linoleic, methyl oleate and methyl stearate, respectively. Nevertheless, there is glycerol which is also produced as the by-product and is placed separately from biodiesel; hence the purification process still needs to be done.

During purification process, the mix of biodiesel products was firstly added to water, which increases separation of glycerol from oil phase. This method is very effective for lowering the glycerol content in biodiesel. Based on the analysis results, the amount of glycerol in biodiesel before separation process does not meet ASTM D6751 and RSNI EB 020551 standards (around 0.3014 ± 0.05 %). The standards define maximum glycerol value in biodiesel being 0.24 % [21]. After proceeding the purification process with glutaraldehyde-crosslinked chitosan membrane for 1 h, the glycerol content in biodiesel is reduced to 0.1429 ± 0.09 % and meets ASTM D6771 standard. In the process of purifying biodiesel, only glycerol would pass through the pores of the membrane due to the size of the glycerol molecules, which are smaller than molecules of biodiesel components. The viscosity of biodiesel at 313 K is 4.8 CST with flash point of 439 K and cloud point of 279 K. These values meet the Indonesian standards, which state that minimum flash point of biodiesel is 373 K and the maximum cloud point is 291 K. The obtained data ensure that chitosan membrane may be useful for enhancing biodiesel quality to meet the standards ASTM D6751 and RSNI EB 020551.

4. Conclusions

Glutaraldehyde-crosslinked chitosan membrane was successfully synthesized from shrimp shell with maximum performance at 3.1 % chitosan concentration and glutaraldehyde concentration of 0.05 %. This membrane has been successfully applied for the biodiesel purification and can reduce the glycerol content in biodiesel to 0.1429 %. The performance of the membranes to separate glycerol from biodiesel shows rejection percentage (R) of 52.58 % and Flux of 45.09 W/m^2 . Characteristics of biodiesel produced from CI seed oil purified with glutaraldehyde-crosslinked chitosan membrane contains alkyl ester (94.133 %) composed of methyl palmitate (18.367 %), methyl linoleate (20.298 %), methyl oleate (35.432 %) and methyl stearate (20.036 %). Resulting biodiesel has glycerol content about 0.1429 %, viscosity value about 4.8 CST, flash point of 439 K and cloud point of 279 K.

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ЗАСТОСУВАННЯ ГЛУТАРАЛЬДЕГІД-ЗШИТИХ ХІТОЗАНОВИХ МЕМБРАН З ВІДХОДІВ ПАНЦИРІВ КРЕВЕТОК ДЛЯ ВИРОБНИЦТВА БЮДИЗЕЛЮ З КАЛОФІЛЕВОЇ ОЛІЇ


Анотація. Для поліпшення якості і зниження вартості виробництва біодизельного палива проведено його очищення з використанням глутаральдегід-зшитих хітозанових мембран. Біодизель одержано перестерифікацією калофілевої олії з використанням кислотного каталізатора. Показано, що кількість гліцерину в біодизельному паливі перед процесом очищення становить $0,3014 \pm 0,05$ %. Після проведення очищення протягом 1 год. вміст гліцерину в біодизелі зменшується до $0,1429 \pm 0,09$ %.

Ключові слова: біодизель, гліцерин, зшивання, хітозан, мембрана.

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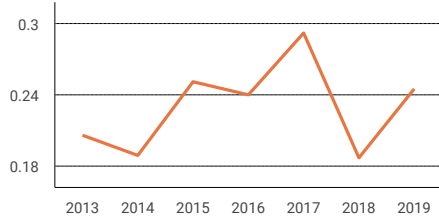
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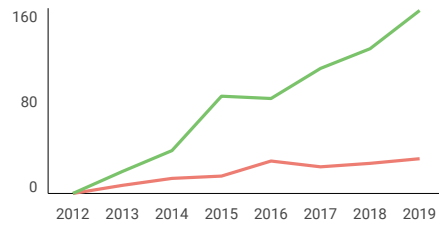
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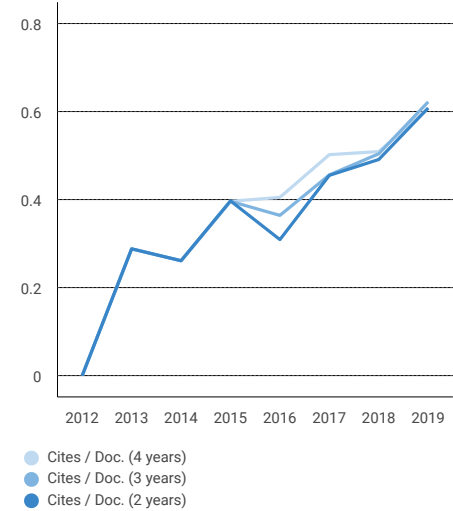


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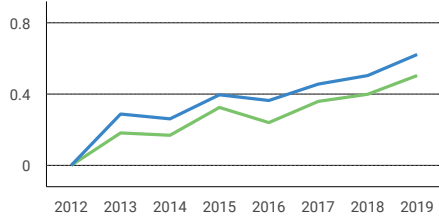


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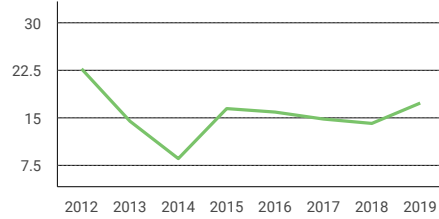


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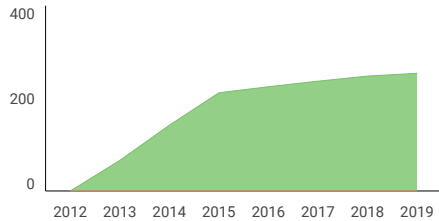


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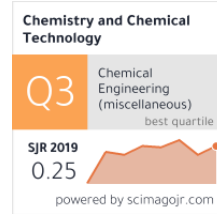
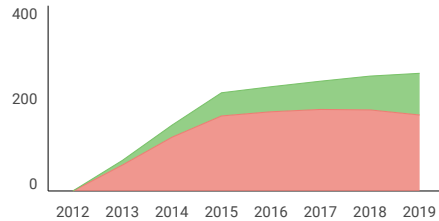
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Tamar kanchaveli

reply

**Melanie Ortiz** 3 months ago

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Melanie Ortiz 6 months ago

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K **Kwenzekile Bhengu** 6 months ago

Hi please help me, I had a Bachelor of Science- Chemical and Chemistry Technology, I looking for internship/ learnership

reply



Melanie Ortiz 6 months ago

SCImago Team

Dear Kwenzekile,
Thank you for contacting us. Could you please expand a little bit on your comment?
Best Regards, SCImago Team

M **Muhabbat Honkeldieva** 1 year ago

I am Muhabbat Honkeldieva and I work at the Institute of Bioorganic Chemistry of Uzbek Academy of Sciences. I am research worker in the field bioorganic and physical chemistry. I want to publish an article in your journal called "Chemistry and Chemical technology". So, how I can get instruction of guideline for authors.

Can you send me all instruction of journal.

The best regards
Muhabbat Honkeldieva
PhD of Chemistry
Institute of Bioorganic Chemistry
Uzbek Academy of Sciences

reply



Melanie Ortiz 1 year ago

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Dear Muhabbat,
thank you for contacting us.
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Best Regards, SCImago Team

N **Nanik AR** 2 years ago

Warm regards

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Thank you

Best regards
Nanik AR

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Elena Corera 2 years ago

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1 message

Olena Shyshchak <oshyshchak@gmail.com>
To: Mochamad Zakki <m.zakki.fahmi@fst.unair.ac.id>

Tue, Aug 15, 2017 at 6:00 PM

Dear authors,

We received your manuscript, thanks, and sent it for the review (registration number is 060/13.07.17). After receiving referee report I'll send it to you for comments. Please, nitoe that the period of time from submitting paper to its publication is approximately 6-8 months.

Best regards,

Dr. Olena Shyshchak

Executive secretary

journal Ch&ChT



oshyshchak@gmail.com



99+

Fw: Re: Re: Revised Manuscript_Siti Wafiroh



siti wafiroh <sitiwafiroh@yahoo.com>

to me

----- Forwarded Message -----

From: siti wafiroh <sitiwafiroh@yahoo.com>

To: Alfa Akustia <alfaakustia@gmail.com>

Sent: Friday, October 20, 2017, 9:48:01 AM GMT+7

Subject: Fw: Re: Re: Revised Manuscript_Siti Wafiroh

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From: Olena Shyshchak <oshyshchak@gmail.com>

To: siti wafiroh <sitiwafiroh@yahoo.com>

Sent: Thursday, September 7, 2017, 12:27:32 PM GMT+7

Subject: Re: Re: Revised Manuscript_Siti Wafiroh

Thanks

2017-09-07 5:39 GMT+03:00 siti wafiroh <sitiwafiroh@yahoo.com>:

Dear Olena Shyshchak,

Thank you for your attention, this is my postal address:

Siti Wafiroh, S.Si.M.Si.

Department of Chemistry

Faculty of Science and Technology

Airlangga University

Campus C Mulyorejo Surabaya (60115) call, fax +62(031)5922427



oshyshchak@gmail.com



99+

From: Siti Wafiroh <sitiwafiroh@yahoo.com>

To: Alfa Akustia <alfaakustia@gmail.com>; Alfa Akustia Widati <alfaakustia@fst.unair.ac.id>

Sent: Thursday, August 31, 2017, 9:58:30 AM GMT+7

Subject: Fw: your paper in Ch&ChT

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From: Olena Shyshchak <oshyshchak@gmail.com>

To: "sitiwafiroh@yahoo.com" <sitiwafiroh@yahoo.com>

Sent: Wednesday, August 30, 2017, 2:42:48 PM GMT+7

Subject: your paper in Ch&ChT

Dear authors,

I am preparing now your article for the final acceptance and need some corrections. All my questions and send me back also marked with the color.

Best regards,

Olena Shyshchak

Executive secretary

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