Investigation of the Use of Ceramic Membranes in Recovering Liquid Enzymes for Castor Oil Transesterification

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The reuse of the liquid enzymes Eversa Transform and Resinase HT for castor oil transesterification was investigated. For each enzyme, the reactions were carried out at 35 °C with 6:1 methanol-to-oil molar ratio, 5 wt% enzyme and 5 wt% water by weight of oil, for 8 hours. After completion, the reaction mixture was centrifuged at 4000 rpm for 30 min and the enzyme phase was collected. The reuse of the enzyme solutions were tested using three different separation techniques. In the first, enzymes were reused after the centrifugation step without further separation. In the second, enzymes were reused after centrifugation followed by water diafiltration using an ultrafiltration membrane in order to remove glycerol and methanol from the recycle solution. In the third, the enzyme solution obtained after diafiltration was concentrated in the membrane set-up prior to its reuse. Tubular ceramic ultrafiltration membranes with MWCO of 15 and 25 kDa were used.

The experiments showed that ultrafiltration removed glycerol and methanol from the enzyme solution, increasing the enzyme activity during the transesterification. Furthermore, concentration of the enzyme solutions after the diafiltration had a positive effect on the FAME production when recycled enzyme solutions are used. For Eversa Transform, the biodiesel content increased from 67 % with reuse after centrifugation to 83 % when a concentrated and diafiltrated enzyme solution was used as catalyst. For Resinase HT, the biodiesel concentration improved from 69 to up to 79 %.

1. Introduction

The search for alternative renewable fuels to replace the use of fossil ones makes biodiesel production a field of continuous interest. Biodiesel is generally produced by transesterification of vegetable oils or animal fats with short chain alcohols such as methanol and ethanol. Enzyme-catalyzed transesterification is an alternative that can overcome the drawbacks associated with the use of chemical catalysts (Guldhe, et al., 2015). The enzyme-catalyzed process can operate under mild reaction conditions; can handle a larger variety of feedstocks and gives easier product separation after the reaction. Besides that, it is less energy-intensive and more environmentally friendly (Christopher et al., 2014). However, the main disadvantage is the higher cost of enzymes compared to chemical catalysts. Enzyme reuse is a possibility that could reduce the operating costs. Enzymes are commercially available in liquid and immobilized forms. Even though immobilized enzymes are more stable and easily handled, they are more expensive than liquid enzymes (Fjerbaek et al., 2009). However, the use of liquid enzymes is limited by the difficulty of recovering them from the reaction medium. Enzyme inhibition is caused by the presence of glycerol, leading to a decrease in the biodiesel yield (Nielsen et al., 2008). The reuse of liquid enzymes has been shown to result in a consistent high yield over multiple batch runs using a mixture of 50 % recovered and 50 % fresh enzymes. However, in the case of full reuse of recovered enzymes, the yield continuously decreases (Andrade et al., 2017).

Membrane technology emerges as an alternative to purify the liquid enzyme solutions. Membrane separation for the purification and concentration of components is still considered an emerging unit operation. The application of membrane technology achieves a high efficiency without addition of chemicals, has a low energy requirement and is easy to handle (Padaki et al., 2015).

Membranes are defined as semipermeable barriers that separate two phases and restrict the transport of the substances in a specific way. This selective barrier allows the passage of certain substances in the stream called permeate, while other components of the mixture are retained in the retentate. The selectivity of the membrane is related to the membrane pore size and the dimension of the molecules of interest for separation. The membrane barrier is generally a thin, nonporous, polymeric film, but may also be porous polymer, ceramic, or metal materials (Coutinho et al., 2009; Seader and Henley, 2006).

Membrane separation mainly includes microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO). The key difference between these processes is the surface pore size of the membranes and molar masses of the separated components that define their application. UF membranes are characterized by a nominal molecular weight cut-off (MWCO), defined as the smallest globular solute molecular weight in which at least 90 % rejection is obtained by the membrane. UF membranes separate particles with molar masses between 1 and 300 kDa. (Alzahrani et al., 2014; Coutinho et al., 2009; Padaki et al., 2015).

The use of ceramic membranes have several advantages compared to organic membranes in terms of high-temperature durability, sufficient mechanical strength, chemical inertness, organic solvent resistance, unique surface characteristics and less likelihood of bacteria contamination. (Cheng et al., 2009; Zsirai, et al., 2016). Inorganic ceramic membranes are used in MF and UF applications. These microporous membranes are typically made from aluminum, titanium or silicon oxides (Baker, 2012).

The use of UF membranes has previously been investigated in processes for biodiesel wastewater treatment (Delcolle et al., 2017). However, there is a lack of study regarding the recovery of liquid enzymes using membranes. Therefore, cross-flow filtration with ceramic membranes was proposed to evaluate the enzyme recovery in order to reuse in the transesterification reactions. According to information from the manufacturer, the molecular weight for both enzymes used (Eversa Transform and Resinase HT) is close to 32 kDa. Based on the enzymes molecular weight, ultrafiltration membranes were tested to recover the liquid enzymes used for biodiesel production. The experiments intended to eliminate the inhibitors glycerol and methanol from the enzyme-rich phase in order to increase the enzyme activity during the transesterification. Insoluble methanol droplets cause denaturation of the enzyme, while glycerol molecules form a hydrophilic environment around the enzyme, creating mass transfer limitations that prevent the hydrophobic substrate to be exposed to the enzyme (Christopher et al., 2014; Guldhe, et al., 2015).

2. Materials and Methods

2.1 Materials

Ecological castor oil was purchased from Urtegaarden ApS (Denmark). Liquid enzymes Eversa Transform (ET) and Resinase HT (RHT) were kindly provided by Novozymes A/S (Denmark). Methanol, n-hexane, acetonitrile, and isopropanol of HPLC grade were purchased from Sigma-Aldrich. HPLC calibration standards including methyl esters (ricinoleate, linoleate and oleate) and fatty acids (ricinoleic, linoleic and oleic acid) of a 99 % purity grade were also acquired from Sigma-Aldrich. Standards of tri-, di-, and monoglycerides, as well as ethyl ricinoleate were obtained by transesterification and separation on a preparative HPLC.

The ceramic membranes used were UF 15 kDa (Batch no.: 267452) and UF 25 kDa (Batch no.: 267450) from Atech Innovations GmbH. These membranes were tubular made of TiO_2, ZrO_2 and Al_2O_3. The membrane's length and thickness were 8 cm and 2 mm, respectively, with an approximate filter surface per element of 15 cm^2.

2.2 Experimental Procedure

Reactions were carried out at 35 °C with 6:1 methanol-to-oil molar ratio, 5 wt% enzyme and 5 wt% water by weight of oil in a 100 mL round-bottom flask equipped with a water-cooled condenser system. The reactor was immersed in a thermostat oil bath equipped with a magnetic stirrer. The reactions were performed for 8 hours at 750 rpm. Methanol was added to the system in four stepwise additions at two-hour intervals, in order to minimize alcoholic enzyme inhibition.

After the reactions, the reaction mixture was centrifuged in a Thermo Scientific Sorvall ST 16R Centrifuge at 4,000 rpm for 30 min and the enzyme-rich phase was collected. The centrifugation step was necessary since the castor oil and esters of castor oil in the membrane setup would increase the viscosity of the feed fluid, decreasing the permeate flux.

Ultrafiltration was carried out in a MiniMem Membrane Separation Lab Unit (PS Prozesstechnik GmbH). Diafiltration in batch mode using the Control Unit for Automatic Diafiltration and Continuous Operation (PS Prozesstechnik GmbH) was coupled to the membrane separation system, using ceramic membranes with MWCO of 15 and 25 kDa. Figure 1 shows the set-up used for the separation. The feed tank contained the enzyme-rich phase obtained from the centrifugation after the transesterification. Feed was continuously mixed with a magnetic stirrer at 200 rpm. Water was used as the diafiltration liquid and added into the feed tank

using a diaphragm pump. The water flowrate was controlled by a level sensor that ensured a constant volume of 40 mL in the feed tank. An HPLC pump was used to pump the feed to the tubular ceramic membrane module. The feed flow rate was kept constant in 10 mL min^{-1}. A venting valve was coupled to the system to prevent the HPLC valve from overpressure (Roda-Serrat et al., 2015). A spring valve was used to regulate the pressure in the membrane module. The retentate was recycled back to the feed tank through the spring valve, while the permeate was collected in the permeate tank, with continuous monitoring of the permeate flux. Filtration occurred at room temperature (24 °C). The transmembrane pressure was kept at 10 bar in order to avoid adsorption of the solute in the pores which would lead to membrane fouling and cause a very low permeate flux.
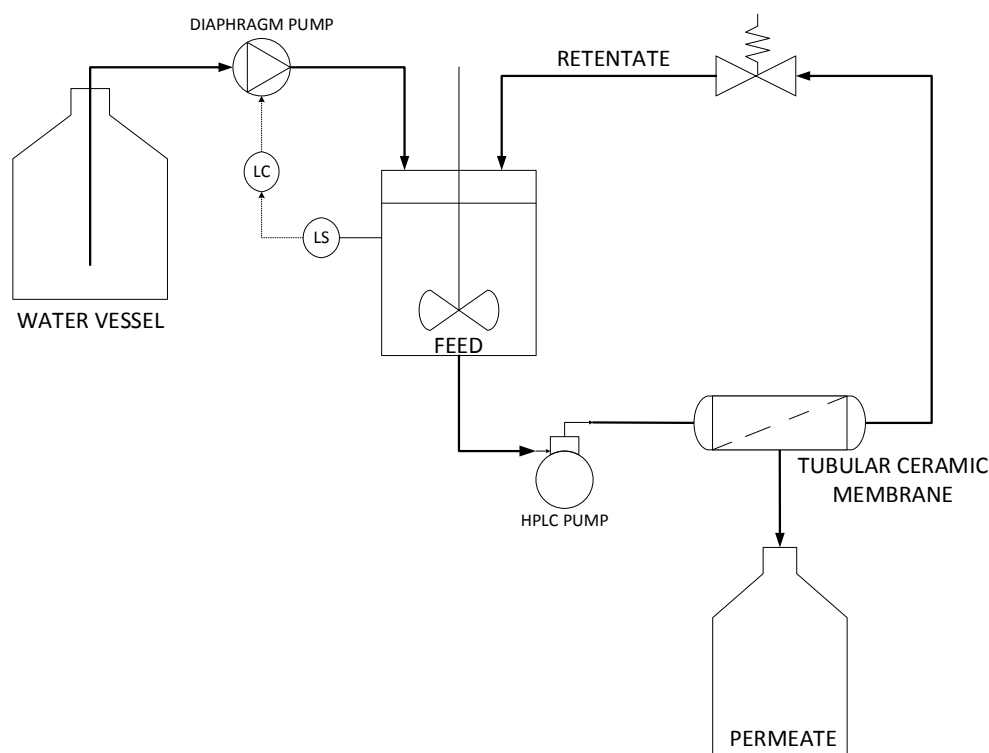

Figure 1: Process set-up for the purification of liquid enzymes by diafiltration and ultrafiltration.

Glycerol and methanol concentrations were determined each hour by an Agilent 1100 Series HPLC equipped with a Phenomenex Rezex RHM-Monosaccharide H+, 300 x 7.8 mm column and a Refractive Index Detector (RID). Water was used both as solvent and mobile phase with a flow rate of 0.6 mL/min. The runtime for the analysis was 25 min, with temperatures of 80 and 40 °C for the column and RID, respectively.

After glycerol and methanol had been removed, the process was operated as an ordinary ultrafiltration in order to concentrate the feed mixture. The feed weight was reduced to 30 % of its initial weight by UF in the Eversa Transform solution and to 45 % in the Resinase HT solution. The water mass separated in each permeate corresponded to the approximate amount of glycerol and methanol in the enzyme-rich phases before the diafiltration step. After each filtration, the set-up was cleaned with deionized water for 10 min, followed by a 20 min flush with a cleaning solution of 0.01 % NaOH and finally rinsed for 10 min with deionized water to adjust the membrane pH back to neutral.

Reuse of the enzymes was tested according to the procedure shown in Figure 2. Transesterification reactions were carried out under the same reaction conditions used when fresh enzymes catalyzed the reaction (A). Enzyme solutions reused in the following reactions were recovered according to three different separation processes. First, enzymes recovered from the centrifugation were directly reused in the transesterification (B). Secondly, the enzyme-rich phase obtained from the centrifugation was purified by means of diafiltration. The enzyme solution free of methanol and glycerol obtained in the feed tank immediately after the diafiltration was reused as the reaction catalyst (C). Finally, enzyme solution obtained from the diafiltration was concentrated in the membrane set-up with water removal prior to its reuse in the reaction (D).

Figure 2: Enzymes conditions and process separation used for the enzyme recovery for reuse.

3. Results and Discussion

Figures 3 and 4 show the amount of glycerol and methanol present in the feed for the diafiltration of Eversa Transform and Resinase HT, respectively. After the centrifugation, the ET-rich phase contained around 52 wt% glycerol and 21 wt% methanol, while the RHT-rich phase contained 29 wt% glycerol and 25 wt% methanol. For the purification of Eversa Transform, at least 9-hour diafiltration was necessary to guarantee high glycerol and methanol removal. Conversely, 4-hour of diafiltration was enough to reduce the amount of glycerol and methanol to less than 1 wt% from the enzyme-phase in the purification of Resinase HT.
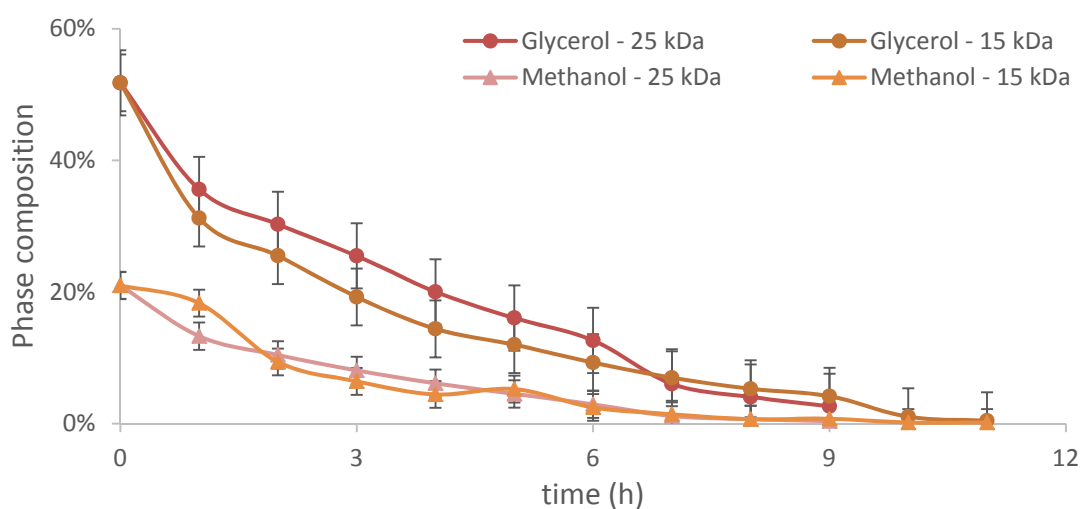

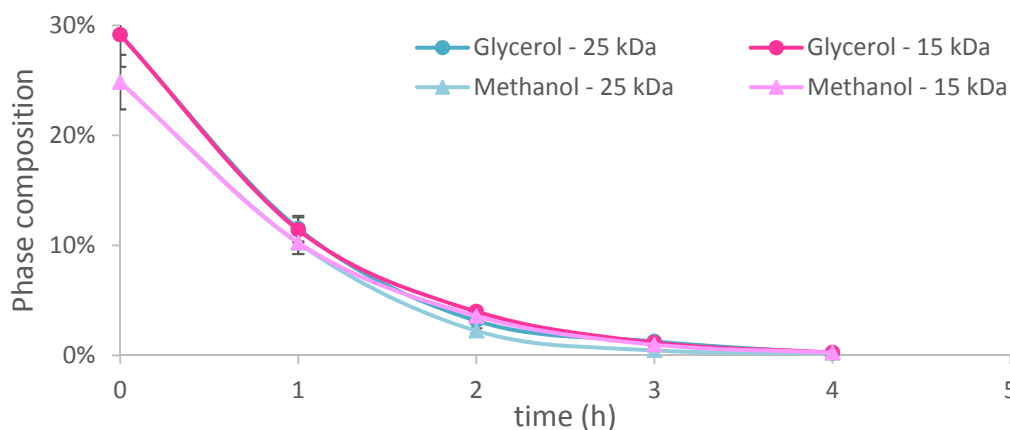*Figure 3: Glycerol and methanol amounts in the Eversa Transform-rich phase for the diafiltration.*

Figure 4: Glycerol and methanol amounts in the Resinase HT-rich phase for the diafiltration.

The variation in the permeate flow of each diafiltration is shown in Figure 5. Dilution with a nearly constant flux was obtained for the diafiltration of the solutions containing ET enzyme. For the solutions with RHT, the permeate flux increased after one hour of filtration. This increase was probably due to the high viscosity of the feed at initial conditions. After this period, the permeate flux remained approximately constant.
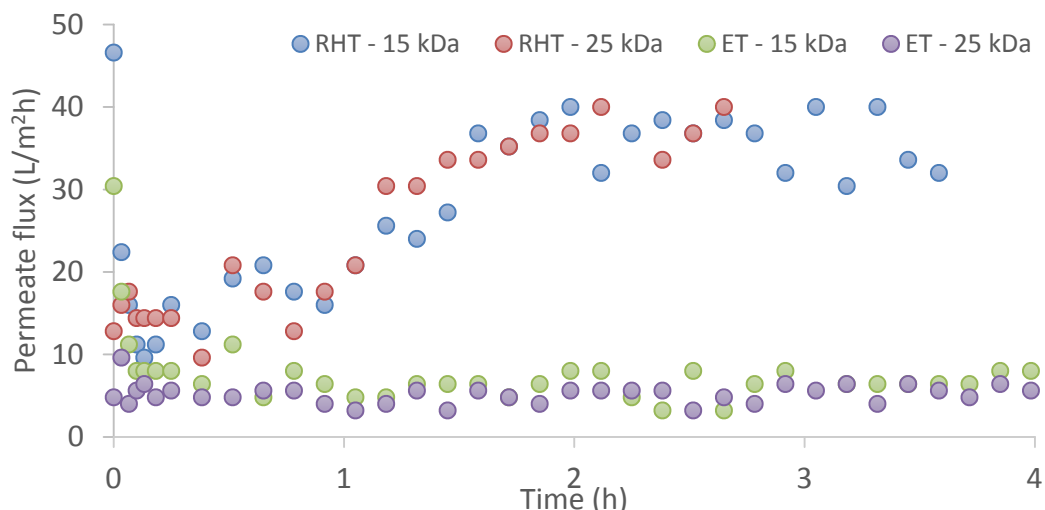
Figure 5: Permeate flow for diafiltration with ceramic membrane of different MWCO.

Regarding the reduction of the amount of these substances with time of diafiltration, comparable behaviors were observed for the use of both membranes, which suggests that both ceramic membranes have similar efficiencies to separate glycerol and methanol from the reaction mixture.

The molar composition of the oil-biodiesel phase for each enzyme reuse condition after 8-hour reaction is shown in Table 1. After catalysis with fresh enzymes (A), the molar composition of the oil-biodiesel phases with both ET and RHT catalysts was of around 94 % FAME and 6 % FFA. Reduction in the FAME content was observed when the liquid enzymes were reused after the centrifugation (B). In this case, FAME content was in the range of 67 – 69 %, while 10 % FFA was obtained. In addition to the higher FFA content, the presence of unreacted glycerides (TAG, DAG and MAG) indicated that transesterification and esterification reactions were not complete. This fact was probably because of the loss of the enzymes' activity after the first batch, and the presence of glycerol and methanol that leads to enzyme inhibition.

Table 1: Molar composition of the oil-biodiesel phase after 8 hours of transesterification reactions using enzymes recovered from different separation processes.

Enzyme	Separation		MWCO (kDa)	Oil-biodiesel composition (%)				
				TAG	DAG	MAG	FAME	FFA
ET	A	Fresh enzyme	-	0.0±0.0	0.0±0.4	0.0±0.7	94.0±1.1	6.0±0.2
	B	Centrifugation	-	0.0±0.0	4.3±0.1	18.5±0.6	67.2±2.0	10.0±0.3
	C	Diafiltration	15	0.0±0.0	3.5±0.1	14.4±0.4	67.6±2.0	14.5±0.4
			25	0.0±0.0	3.9±0.1	16.9±0.5	64.1±1.9	15.1±0.5
	D	Concentration	15	0.0±0.0	0.0±0.0	7.1±0.2	82.8±2.5	10.1±0.3
			25	0.0±0.0	0.0±0.0	7.1±0.2	82.7±2.5	10.2±0.3
RHT	A	Fresh enzyme	-	0.0±0.0	0.0±0.0	0.0±0.0	93.8±2.8	6.2±0.2
	B	Centrifugation	-	0.9±0.0	4.0±0.1	16.3±0.5	68.7±2.1	10.1±0.3
	C	Diafiltration	15	2.7±0.1	5.1±0.2	23.6±0.7	54.2±1.6	14.4±0.4
			25	1.3±0.0	4.2±0.1	18.6±0.6	62.5±1.9	13.4±0.4
	D	Concentration	15	0.0±0.0	0.0±0.0	10.0±0.3	78.9±2.4	11.1±0.3
			25	0.0±0.0	3.2±0.1	12.1±0.4	72.8±2.2	11.9±0.4

Enzyme reuse after the diafiltration showed no improvement in the FAME production (C). For the reuse of ET, FAME content was similar to when the enzyme was reused after centrifugation. The reuse of RHT after diafiltration resulted in lower FAME content than after centrifugation. For both enzymes, even though glycerol and methanol were removed from the solution in the permeate stream, the water content increased, leading to higher hydrolysis of castor oil and, therefore, the FFA content increased from 10 to up to 15 %.

Concentration of the enzyme solutions after the diafiltration (D) had a positive effect on the FAME content when the enzymes were reused. In case of reuse of ET, the FAME content increased to around 83 %, while up to 79 % FAME was observed for the reuse of RHT. Removal of water in this process increased the enzyme

concentration in the solution and reduced the hydrolysis competition with transesterification, generating lower FFA content.

For the enzyme reuses after both diafiltration and concentration steps, similar FAME contents were obtained for the use of ceramic membranes with MWCO of 15 and 25 kDa. This indicated that the enzyme retention for both membranes was similar. However, thorough investigation is recommended to reach that conclusion, such as measuring the enzyme concentration in both retentate and permeate solutions, and evaluating the ultrafiltration under different filtration conditions, such as transmembrane pressures and membrane pore sizes.

4. Conclusions

UF ceramic membranes were able to eliminate glycerol and methanol from the enzyme-rich phase of Eversa Transform and Resinase HT solutions. By using diafiltration the glycerol and methanol content were reduced to less than 1 wt%. After concentration of these enzyme solutions, the enzyme reuse resulted in higher biodiesel production, increasing the FAME content from 67 to 83 % when reusing Eversa Transform, and from 69 to up to 79 % FAME content when reusing Resinase HT. The use of membrane technology for recovery of liquid enzymes is thus promising. However, further investigation is required to increase the efficiency of the separation.

Acknowledgments

References

Alzahrani S., Wahab A., 2014, Challenges and trends in membrane technology implementation for produced water treatment: A review, Journal of Water Process Engineering, 4, 107–133.

Andrade T.A., Errico M., Christensen K.V., 2017, Castor Oil Transesterification Catalysed by Liquid Enzymes: Feasibility of Reuse under Various Reaction Conditions, Chemical Engineering Transactions, 57, 913–918.

Baker R.W., 2012, Membrane Technology and Applications, 3rd ed., John Wiley & Sons, Inc. Chichester, UK.

Cheng L.H., Cheng Y.F., Yen S.Y., Chen J., 2009, Ultrafiltration of triglyceride from biodiesel using the phase diagram of oil-FAME-MeOH, Journal of Membrane Science, 330, 156–165.

Christopher L.P., Kumar H., Zambare V.P., 2014, Enzymatic biodiesel: Challenges and opportunities, Applied Energy, 119, 497–520.

Coutinho C.M., Chiu M.C., Basso R.C., Ribeiro A.P.B., Gonçalves L.A.G., Viotto L.A., 2009, State of art of the application of membrane technology to vegetable oils: A review, Food Research International, 42, 536–550.

Delcolle R., Gimenes M.L., Fortulan C.A., Moreira W.M., Martins N.D., Pereira N.C., 2017, A Comparison between Coagulation and Ultrafiltration Processes for Biodiesel Wastewater Treatment, Chemical Engineering Transactions, 57, 271–276.

Fjerbaek L., Christensen K.V., Norddahl B., 2009, A review of the current state of biodiesel production using enzymatic transesterification. Biotechnology and Bioengineering, 102, 1298–1315.

Guldhe A., Singh B., Mutanda T., Permaul K., Bux F., 2015, Advances in synthesis of biodiesel via enzyme catalysis: Novel and sustainable approaches, Renewable and Sustainable Energy Reviews, 41, 1447–1464.

Nielsen P.M., Brask J., Fjerbaek L., 2008, Enzymatic biodiesel production: Technical and economical considerations, European Journal of Lipid Science and Technology, 110, 692–700.

Padaki M., Surya Murali R., Abdullah M.S., Misdan N., Moslehyani A., Kassim M.A., Hilal N., Ismail A.F., 2015, Membrane technology enhancement in oil-water separation. A review, Desalination, 357, 197–207.

Roda-Serrat M.C., Christensen L.P., Christensen K.V., Fretté X., 2015, Optimization of removal of D-trehalose from commercial food colorant by diafiltration with ultrafiltration membranes, Chemical Engineering Transactions, 43, 1027–1032.

Seader J.D., Henley E.J., 2006, Separation process principles, 2nd ed., John Wiley & Sons, Inc., USA.

Zsirai T., Al-Jaml A.K., Qiblawey H., Al-Marri M., Ahmed A., Bach S., Watson S., Judd S., 2016, Ceramic membrane filtration of produced water: Impact of membrane module, Separation and Purification Technology, 165, 214–221.