Desalination and Water Treatment
www.deswater.com

doi:10.5004/dwt.2017.21711

98 (2017) 169–175
December

An *in-situ* transesterification of municipal activated sludge for biodiesel production

Hoang-Nhat-Phong Vo[a], Xuan-Thanh Bui[b,*], Thanh-Tin Nguyen[c,d], Tan-Phuoc Nguyen[b], Thi-Hong-Hanh Le[c], Thanh-Son Nguyen[c], Thi-Dieu-Hien Vo[e], Chitsan Lin[e]

[a]*Environmental Engineering and Management Research Group & Faculty of Environment and Labour Safety, Ton Duc Thang University, Ho Chi Minh City, Vietnam, email: vohoangnhatphong@tdt.edu.vn (H.-N.-P. Vo)*
[b]*Faculty of Environment and Natural Resources, University of Technology, Vietnam National University – Ho Chi Minh, No. 268 Ly Thuong Kiet Street, District 10, Ho Chi Minh City, Vietnam, email: bxthanh@hcmut.edu.vn (X.-T. Bui), btnt06@hotmail.com (T.-P. Nguyen)*
[c]*Institute of Research and Development, Duy Tan University, 03 Quang Trung, Da Nang, Vietnam, email: thanhtin201@yahoo.com (T.-T. Nguyen), honghanh.mt6c@gmail.com (T.-H.-H. Le), ntson@hotmail.com (T.-S. Nguyen)*
[d]*Faculty of Food and Environment, Dong Nai Technology University, Dong Nai, Vietnam*
[e]*Institute of Marine Science and Technology, National Kaohsiung Marine University, Kaohsiung city 81157, Taiwan, email: hien.ic.tracodi@gmail.com (T.-D.-H. Vo), ctlin@mail.nkmu.edu.tw (C. Lin)*

Received 4 July 2017; Accepted 22 November 2017

ABSTRACT

Activated sludge is a potential feedstock for biodiesel production with co-benefit in waste remediation and sustainable energy compared to other food-based materials. In this research, the wasted activated sludge was examined with *in-situ* transesterification process. The optimal mass ratio of methanol to sludge, H_2SO_4 catalyst concentration (v/v to methanol) and temperature were investigated as 8:1, 5% and 75°C, respectively. The highest yield was observed at 8 h of retention time being 5.28%. Three most popular fatty acid methyl esters (FAMEs) were palmitic acid (C 16:0 with 27.76% mol/mol FAMEs), oleic acid (C 18:1 with 19.30% mol/mol FAMEs) and palmitoleic acid (C 16:1 with 16.93% mol/mol FAMEs). The optimal extraction conditions were in congruent with other studies with promising FAMEs yield, implying surplus fatty acid content of this local feedstock. The physio-chemical properties of FAMEs needed further improvement to please the standard and commercialization requirement.

Keywords: Biodiesel; In-situ transesterification; Municipal activated sludge; Fatty acid methyl esters (FAMEs); Free fatty acids (FFAs)

1. Introduction

The indiscriminate exploitation of fossil fuel has been leading to the energy crisis in recent years. Meanwhile, the increasing consumption of fossil fuel is condemned for amounts of environmental problems. This urges for the novel renewable source which can satisfy adverse effects on environment and slow down the depletion of fossil fuel. Biodiesel, compressed natural gas (CNG), liquefied petroleum gas (LPG) and reformulated gasoline have been suggested as replaceable ones. Of these alternatives, biofuel is a non-fossil source and researcher has stated its competitive quality to diesel fuel and minor requirements for modification prior to use [1].

Biodiesel is biodegradable, renewable and safer than diesel fuel as it contains less sulfur ingredient. The generated contaminants (i.e. hydrocarbons, carbon monoxide and particulate matters) are significantly less, reported by several researches [2]. However, the application of biodiesel in diesel engines associated with some problems. The engine deposit, injector coking and piston ring sticking

*Corresponding author.

might happen due to its high viscosity and low volatility [2]. This is tackled by transesterification process to remove glycerin from triglycerides and obtain FAMEs. This result in the decrease of viscosity as well as the cetane number and heating value are unchanged [3].

Biodiesel has been traditionally derived from vegetable oils or animal fats such as date palm seed [4], rape seed and soybean [5]. However, the cultivation of these materials requires an extensive land area. Statistics show that the raw materials account for 75% of total biodiesel production [3] as well as the concerns for famine risk [6] because these resources supplied for industrial purpose. The demand for low cost feedstock, consequently, becomes urgently need.

The treatment of domestic wastewater by activated sludge processes generates large amounts of wasted activated sludge (WAS). This kind of biodegradable material was legislated in European Union (directive on landfilling waste 1999/31/EC) [7] for its stability and health risk issues. The reuse of activated sludge, therefore, serves as a sustainable alternative fulfilling the demands of pollution control and cost efficiency. Composting and nutrient recycling methods were an indispensable ones [8]. An innovative approach which adopt wasted activated sludge for biodiesel production is a promising technique, in term of material availability, energy recovery and pollution control [9].

The lipid content of activated sludge is plentiful stem from the adsorption of domestic and industrial pollutants to the sludge. The contribution of micro-organisms cell membranes, metabolites and by products via cell lysis in term of phospholipids is also huge. Jardé et al. [10] stated the fatty acids of C_{10} – C_{18} constituted for a significant amount in almost municipal activated sludge. These lipids were potential resources for biodiesel production [11]. However, the use of alkaline catalyst in transesterification process could form soap which prevent the separation of biodiesel from glycerin fraction [3]. Some researchers have demonstrated acid catalyst was more adoptable to high fatty acid content feedstock such as activated sludge [12,13].

In this research, the municipal activated sludge in Binh Hung central wastewater treatment plant (CWTP) in Ho Chi Minh city (Vietnam) was used as feedstock for *in-situ* biodiesel production. The extraction parameters (mass ratio of methanol to sludge, acid catalyst concentration (v/v to methanol) and temperature) were optimized for maximum FAMEs yield achievement. The fatty acid profile of FAMEs were also analyzed for further comparison with other feed stocks.

2. Materials and methods

2.1. Used materials

The wet concentrated activated sludge samples were collected from sludge of thickener of Binh Hung central wastewater treatment plant. This wastewater treatment plant treated municipal wastewater (141,000 m^3/d) using conventional activated sludge process. The operating conditions of the aeration tank were F/M ratio of 0.3 g BOD/g MLVSS·day and SRT of 9 d. Dewatered sludge produced with capacity of 35 ton/d. The characteristics of thickened sludge used for this study were 6.55 (pH), 12.48 g/L (total solids) and 7.45 g/L (total volatile solids).

The sludge samples were firstly concentrated by settling at 10°C for 24 h. Afterwards, the wet sludge was centrifuged using Universal 320 Hettich Zentrifugen machine at 4,000 rpm for 15 min. The dewatered sludge was continually dehydrated in natural condition for five days (avoid sunlight exposure). Subsequently, the dried sludge (DS) samples (moisture 10%) were crushed, homogenized and stored in the refrigerator at 5°C for experimental processes.

Methanol, sulfuric acid, n-hexane C_6H_{14} and other reagents were analytical grade for the *in-situ* transesterification experiments.

2.2. Experimental design

In this study, the effect of mass ratio (methanol to sludge), H_2SO_4 catalyst concentration and reaction temperature on FAME yield was examined. The value of these factors was varied and the experimental design was divided into three steps. In the first step, the mass ratio of methanol to DS was investigated in three ratios of 4:1, 8:1, and 12:1. The acid catalyst (H_2SO_4) concentration was adjusted at 5% v/v to methanol, and the reaction temperature was maintained at 75°C. In the second step, the optimized mass ratio of methanol to sludge from step one was experimented coupling acid catalyst concentration at 3% and 7% v/v to methanol (with temperature remain unchanged 75°C). Similarly, the third step was to investigate the optimal temperature at 55°C and 85°C, whereas the mass ratio of methanol to ΔS and acid catalyst were fixed with optimal values in the previous two steps. The FAME extraction yield was examined according to retention time of 0.5, 4, 8 and 16 h.

2.3 Analysis

2.3.1. In-situ transesterification

The *in-situ* transesterification method was obtained from Mondala et al. [14]. The transesterification initiated with 5 g of sludge sample extracted by combinations of mass ratio of methanol to sludge, catalyst concentration and temperature as in optimization experiments. From the first step of optimization experiment, the amount of methanol and sulfuric acid were calculated based on mass ratio (methanol to sludge) and concentration of sulfuric acid to methanol, which were 4:1 and 5%, respectively. These volumes were mixed with sludge sample, kept at ambient temperature and well-mixed by magnetic stirring bar.

Afterwards, a 25 mL volume of hexane was added into sample to improve the lipid solubility of the transesterification process. The mixture was stirred by magnetic bar, connected with condenser at designated temperature in optimization experiment (75°C). The reaction time were 0.5 h, 4 h, 8 h and 16 h for FAME extraction, respectively. The loss of methanol and solvent hexane due to evaporation was minimized by using a condenser with water at ambient temperature (25°C). After reaction, the mixture was cooled, added 5 ml of saturated NaCl solution and 50 ml of hexane. Subsequently, it was centrifuged at 400 rpm for 15 min. The supernatant hexane phase was withdrawn for further extraction while the bottom layer was re-extracted.

The resulting supernatant solution was washed with 10 ml of 2% (w/v) potassium bicarbonate ($KHCO_3$) and settled.

The upper layer was subsequently dried with anhydrous sodium sulfate, filtered and purified by vacuum distillation (Buchi R-20 Rotary Evaporator at 320 m bar and 35–26°C). The aliquot was analyzed for FAME yield and properties.

The extraction process was continued with others mass ratio (methanol to sludge), acid concentration and temperature as in optimization experiments.

The FAMEs yield (%) was calculated by the ratio between fatty acid methyl ester (g) in the product and dried solid in the sludge (g) multiplied with 100.

2.3.2. Analytical methods and determination of FAME properties

The FAMEs extraction was identified by gas chromatography, modified from Mondala et al. [14]. This gas chromatography (GC - Agilent 7890A) equipped with a flame ionization detector (FID) and mass spectrometer detector (5975 inert MSD). The used column was a DBS Agilent (30 m × 0.25 mm × 0.25 µm). The temperature in column was kept at 150°C for 1 min; increased from 150°C to 250°C at 5°C/ min; maintained at 250°C for 1 min; ramped from 250°C to 320°C at 20°C/min and finally kept at 320°C for 15 min. The detector temperature was set at 340°C. The carrier gas was helium He (14 psi) at a flow rate of 0.8 mL/min, while the sample injection volume was 1.0 µL with a split ratio of 100:1. The different FAMEs were identified based on the data obtained from GC-MS runs, comparing retention times with available standard and matching mass spectral data to catalog of standards.

The biodiesel sample was analyzed for its fatty acid profile in Petroleum Laboratory-Ho Chi Minh City University of Technology.

3. Results and discussion

3.1. Effect of methanol to sludge mass ratios

Lipid and oil can be extracted by various solvents as hexane, toluene, ethanol and methanol. The extraction with methanol perform a slightly higher yield of fatty acid compared to other solvents like toluene and hexane [15]. The yield of biodiesel, affected by mass ratio of methanol to ΔS in this experiment, is illustrated in Fig. 1. This, overall, increased according to the length of retention time. The optimal extraction yield was observed at ratio 8:1 within three varieties (i.e. 4:1, 8:1 and 12:1). The retention time between 4 to 8 h obtained the significant yield of mass ratio 8:1, coupling H_2SO_4 concentration 5% (v/v to methanol) and temperature 75°C. The latter exposure time remained stable in yield extraction until 16 h. The lack of fatty acid after 8 h due to remarkable conversion to methyl esters in initial phase responsible for the issue [14]. Some studies experienced the similar optimal reaction time at 8 h with the involvement of co-solvent methanol and hexane at 55°C, methanol to sludge ratio at 10 ml/g [16]. Huynh et al. [17] also stated 8 h was an optimal time for FAMEs extraction with methanol to sludge ratio at 30 ml/g.

The yield efficiency of mass ratio 4:1 was low due to the insufficient amount of methanol; whereas the performance of mass ratio 12:1 was initially promising (at 0.5 h); however, increased slower compared to mass ratio 8:1 in the subsequent phase (after 4 h). The higher methanol concentration of mass ratio 12:1 have led to the more dynamic equilibrium at the first half hour; but remained steady after 4 h due to the abundant of water in high methanol concentration. Encinar et al. [18] and García-Moreno et al. [19] insisted that the redundant of methanol was necessary to lead the equilibrium towards the generation of FAMEs. The finding also mentioned that the overdose of methanol brought difficulty in the separation of glycerol and FAMEs; consequently, decreasing FAMEs yield. Ma and Hanna [20] suggested the water content should be kept below 0.06% (w/w) to optimize the conversion. However, Mondala et al. [14] investigated that FAMEs extraction of primary and secondary sludge were 14.5% and 2.5% with methanol to sludge mass ratio at 12:1. The author insisted that the high FAMEs yield resulted from the mutual effect of other relevant extraction parameters.

With respect to other feed stocks, Ullah et al. [21] stated that the highest biodiesel yield was obtained with 15:1 methanol to oil ratio at 160°C in 60 min as converting waste cooking oil to biodiesel. di Bitonto et al. [22] in the effort to generate FAMEs from municipal wet sewage scum, investigated optimal methanol to sludge ratio was 10:1. The result of this study

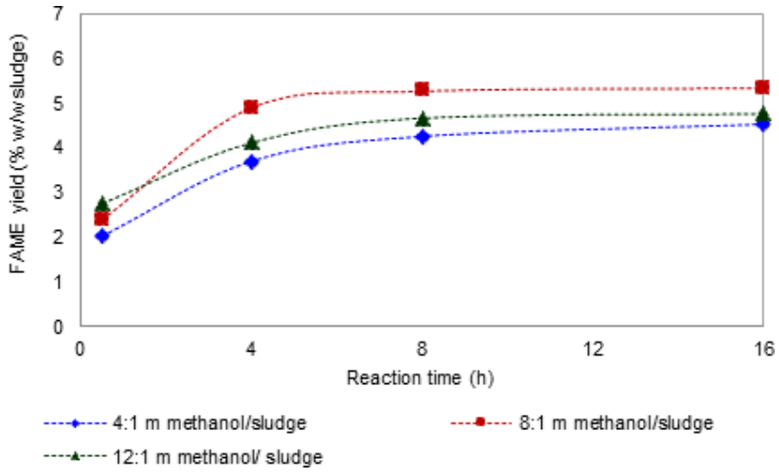

Fig. 1. FAME yield at methanol/sludge mass ratios under 5% (v/v) H_2SO_4 and 75°C.

was slightly lower, at 8:1, due to the different characteristic of feedstock [23]. The adjustment in methanol to sludge ratio was essential to favor the lipid portion of sludge.

3.2. Effect of sulfuric acid catalyst concentration

The free fatty acid (FFA) can be transformed into biodiesel by acid-catalyzed transesterification. The effect of catalyst concentration (H_2SO_4) on FAMEs extraction was apparently similar to methanol to sludge mass ratio shown in Fig. 2. The yield efficiency raised with respect to the length of retention time. This stabilized at 8 h dealing the same reason with mass ratio variation. The maximum FAMEs yield was received at 5% (v/v to CH_3OH) of H_2SO_4. The 7% (v/v to CH_3OH) of H_2SO_4 catalyst demonstrated lower extraction efficiency. The more acidic environment has better lipid solubility, bring in higher FAMEs extraction efficiency [24]. However, the excess of acid component possible cause the degradation of product like 7% (v/v CH_3OH) H_2SO_4 in this case. The higher acid catalyst concentration it was, the higher possibility of polymerization of unsaturated fatty acids/esters happened, causing the depletion of FAME yield [12]. This finding agreed with Tran-Nguyen et al. [25] that the FAMEs yield increased from 25%, 29%, 30%, then decrease to 27%, corresponding to the raise of acid acetic in methanol to acid acetic ratio was 4.75:0.25, 4.5:0.5, 4.25:0.75 and 4.00:1.00, respectively. Mondala et al. [14] concluded the 5% (v/v CH_3OH) H_2SO_4 catalyst gave better extraction efficiency than 1% (v/v CH_3OH) H_2SO_4, with FAMEs yield of minimum 1% and maximum 14%, regardless primary or secondary activated sludge.

It was found that alkaline catalysis could provide faster reaction; however, it had constrain with high free fatty acid feed stock like activated sludge, and required more amount of methanol than acid catalyst [3]. Previous study has demonstrated the universally applied and investigated of H_2SO_4 as catalyst in biodiesel production [26]. The advantages of acid catalyst were remarkable while it was insensitivity to FFAs and water content in oil. Furthermore, the transesterification process was simultaneously and required less energy consumption [27]. Overall, the result of this study was in congruent with other researchers that 5% of H_2SO_4 catalyst was sufficient for FAME yield.

3.3. Effect of reaction temperatures

In this study, the optimal temperature was found at 75°C as shown in Fig. 3. This finding was confirmed by

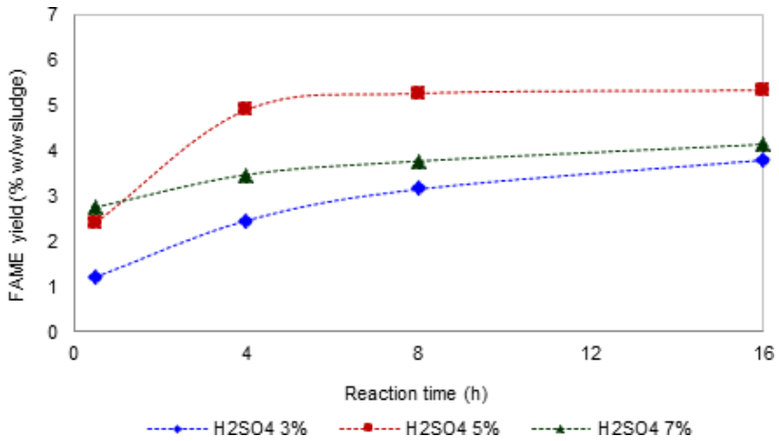

Fig. 2. Effect of H_2SO_4 concentration under 8:1 methanol to sludge mass ratio at 75°C.

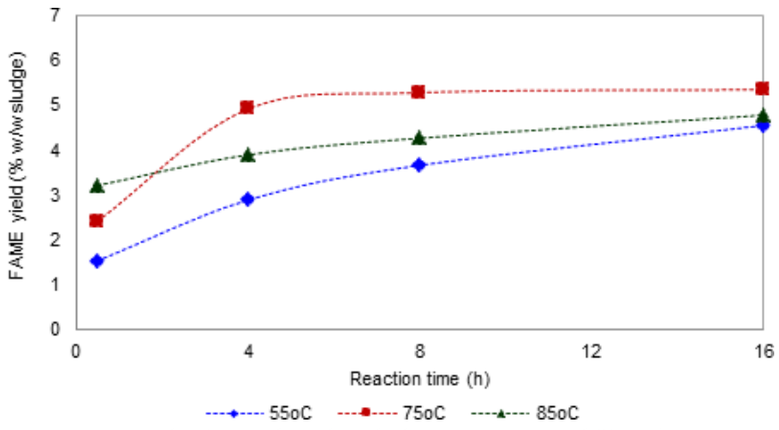

Fig. 3. Effect of reaction temperature under 8:1 methanol to sludge mass ratio and 5% (v/v).

Revellame et al. [12], who conducted a related research with variation of temperature (i.e. 45,55,65 and 75°C) and concluded the temperature above 60°C can decrease the biodiesel yield. The author insisted that the more temperature applied beyond the optimal point, the more yield efficiency reduced due to polymerization of unsaturated fatty acid. On contrary, Amit [28] found the higher temperature increased miscibility of methanol and oil, consequently raised reaction rate, and surged the solubility of fatty acid in methanol. Some research conducted at high temperature as Tran-Nguyen et al. [25] received FAMEs yield 30.11% at 250°C. Huynh et al. [17] got FAMEs yield 7.32% at 200°C and 23.47% at 250°C. However, these authors shortened retention time at 30 min and supposed to face with high energy consumption due to critical extraction temperature. The optimal temperature (75°C) in this research was compatible to retention time at 8 h. The finding was further congruent with some researchers. Mondala et al. [14] investigated optimal condition at 75°C, 5% v/v H_2SO_4, mass ratio methanol to sludge 12:1 after *t*-test data analysis. The reaction temperature ranged from 50°C to 75°C (recommend at methanol boiling point 65°C) with long enough retention time 7–8 h conducted by authors [12,13,16,29].

3.4. Optimization results

The FAME extraction results from other researches are shown in Table 1 with effect of multi-factors in the process. The FAMEs yield is not only the result of these multi-factors but also depends on the lipid content of certain sludge sources. According to Olkiewicz et al. [30], lipid fraction of primary, blended, stabilized and secondary sludge were 25.3%, 21.9%, 10.1% and 9.1%, respectively. The author, however, received limited FAMEs yield extraction of 1% of secondary sludge. Similarly, Mondala et al. [14] got FAMEs yield of 14.5% and 2.5% from primary and secondary activated sludge, respectively. The extraction yield from secondary activated sludge in this study (i.e. 5.28%) was noticeable higher than others; though the optimal condition was not much different. This implies the abundant content of fatty acid and oil in secondary sludge of Binh Hung wastewater treatment plant (WWTP), which possible to reach 35% of sludge [31–33]. Most authors agreed the concentration of catalyst H_2SO_4 was approximately 5% (v/v CH_3OH); coupling temperature 55°C to 75°C. The optimal mass ratio of solvent to sludge, as methanol in this case, fluctuated from 5:1 to 12:1, even reached almost 20:1. The retention time was insisted 8 h.

3.5. FAMEs analysis of biodiesel

Analysis of biodiesel samples in optimal conditions indicated the popular occurrence of methyl esters of palmitic acid (C 16:0), palmitoleic acid (C 16:1) and oleic acid (C 18:1), which accounted for 27.8%, 16.9% and 9.7% (mol/mol FAMEs), respectively (Fig. 4). Dufreche et al. [29], conducting a study in Tuscaloosa WWTP (USA), insisted that these compounds dominated beyond 35%, 15% and 20% (mol/mol FAMEs) of overall FAME products extracted from secondary sewage sludge. The finding of Mondala et al. [14], likewise, stated the methyl esters of palmitic acid (C 16:0), palmitoleic acid (C 16:1) and oleic acid (C 18:1) appeared at a significant level of 35%, 25% and 20%, respectively. In this study, the FAMEs were slightly lower as the different characteristics of activated sludge in Vietnam's WWTP.

The other FAMEs were detected at moderate level, below 5% each. The FAME in Traiguent WWTP (Chile) had a quite similar composition (Table 2), but the percentage was considerably less than Binh Hung and Tuscaloosa WWTPs. This consistently proved the characteristics of sludge strongly affect the FAME ingredient.

Besides triglyceride, fatty acids, phospholipids and bacterial lipids; activated sludge contained numerous amount of chemical substances (i.e. esters, steroids, hydrocarbons, pharmaceutical chemicals), originating from human and industrial activities [10]. These cause difference in biodiesel quality from activated sludge with other feed stocks (i.e. flora and fauna sources). The extraction via *in-situ* transesterification of these compounds made biodiesel quality uncertainty. Specifically, the cloud point of biodiesel product in this study was less than 5. The kinematic viscosity was 0.484 mm^2/s at 40°C which was lower than ASTM D445 standard (1.9–6.0). Also, the carbon residue (w%) was 0.246 and beyond the ASTM D4530 standard (less than 0.05). However, the transformation of these contaminants in activated sludge is expected to extend the fuel yield [34]. This indicated a huge challenge for further research to ensure biodiesel quality. Therefore, this study needs further investigation in biodiesel characteristics and the influence of disturbances in activated sludge to biodiesel yield and quality.

Table 1
Comparison of in-situ transesterification with sulfuric acid catalyst

Mass ratio solvent to sludge (CH_3OH/sludge)	H_2SO_4 catalyst concentration (v/v to CH_3OH)	Temperature (°C)	Retention time (h)	Extraction efficiency (%)	References
8:1	5%	75	8	5.28	This study
5:1	–	64.7	–	3.4	[15]
–	1%	50	–	2.76	[29]
12:1	5%	75	8	2.5	[14]
19.8:1*	4%	55	24	4.79	[12]
7.92:1* (xylene as co-solvent)	5%	55	8	9.68	[16]

Note: DS: dried sludge; WS: wet sludge; *Retrieved from VCH_3OH/m sludge

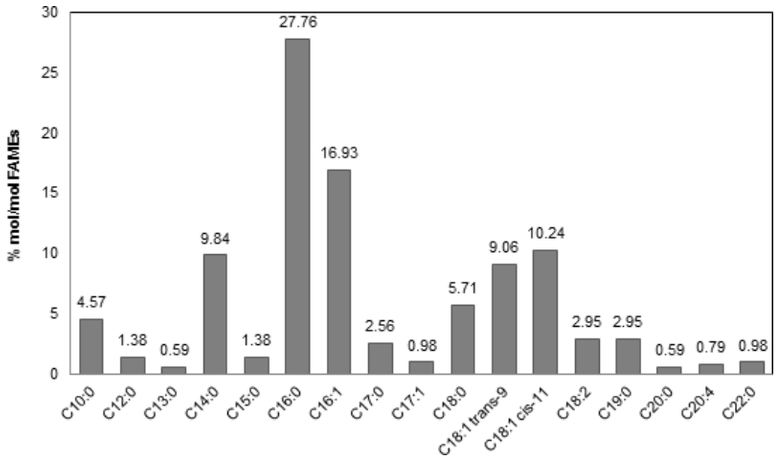

Fig. 4. FAME analysis of biodiesel obtained at 8:1 methanol to sludge mass ratio, 5% (v/v) H_2SO_4, 75°C and 8 h of reaction time.

Table 2
Composition of FAME in different activated sludges

Compositions	Binh Hung CWTP (This study)	Tuscaloosa WWTP (*)	Traiguent WWTP (**)
Solvent	Methanol	Methanol	Methanol
Solid concentration (%)	90	12–14	90
Catalyst	H_2SO_4	H_2SO_4	H_2SO_4
Myristic acid (C14) (%)	9.8	3	1
Palmitic acid (C16:0) (%)	27.8	35	14
Palmitoleic acid (C16:1) (%)	16.9	17	0.5
Stearic acid (C18:0) (%)	5.7	18	6
Oleic acid (C18:1) (%)	9.7	20	1–9
Linoleic acid (C18:2) (%)	3.0	5	3

Remark: Data retrieved from Dufreche et al. [29]* and Sangaletti-Gerhard et al. [13]**

4. Conclusions

In this study, the biodiesel production from activated sludge taken from a sewage central wastewater treatment plant in Ho Chi Minh city (Vietnam) was extracted at optimal conditions via transesterification process. Some concluding remarks are withdrawn as follows:

- The optimal mass ratio of methanol to sludge, H_2SO_4 catalyst concentration (v/v to methanol) and temperature were investigated at 8:1, 5% and 75°C, respectively. This finding mostly agreed with other researches. The extraction FAME yield from this secondary sludge was 5.28%, which noticeably higher than sludge from some other WWTPs.
- The analysis of methyl esters by gas chromatography unveiled three most popular FAMEs were palmitic acid (C 16:0 with 27.76% mol/mol FAMEs), oleic acid (C 18:1 with 19.3% mol/mol FAMEs) and palmitoleic acid (C 16:1 with 16.93% mol/mol FAMEs).
- The characteristic of FAMEs highly depended on compositions of feedstock, the FAMEs extracted from Binh Hung WWTP needed further improvement, in term of physio-chemical characteristics, to please the standard and commercialization requirement.

The results from this research enlighten a potential of activated sludge as a new, cost effective and abundant feedstock for biodiesel production with competitive fuel characteristic. A further research regrading production cost and economic sustainability is highly recommended.

Acknowledgements

Authors would like to acknowledge for research collaboration among Ho Chi Minh City University of Technology, Ton Duc Thang University, Dong Nai Technology University and Duy Tan University, Vietnam.

References

[1] G. Knothe, The Biodiesel Handbook, 2nd ed., AOCS Press, 2010.
[2] M. Lapuerta, O. Armas, J. Rodríguez-Fernández, Effect of biodiesel fuels on diesel engine emissions, Prog. Energ. Combust., 34 (2008) 198–223.

[3] L. Lin, Z. Cunshan, S. Vittayapadung, S. Xiangqian, D. Mingdong, Opportunities and challenges for biodiesel fuel, Appl. Energ., 88 (2011) 1020–1031.
[4] M.W. Azeem, M.A. Hanif, J.N. Al-sabahi, A.A. Khan, S. Naz, A. Ijaz, Production of biodiesel from low priced, renewable and abundant date seed oil, Renew. Energ., 86 (2016) 124–132.
[5] E. Gülşen, E. Olivetti, F. Freire, L. Dias, R. Kirchain, Impact of feedstock diversification on the cost-effectiveness of biodiesel, Appl. Energ., 126 (2014) 281–296.
[6] J. Kansedo, K.T. Lee, Process optimization and kinetic study for biodiesel production from non-edible sea mango (Cerbera odollam) oil using response surface methodology, Chem. Eng. J., 214 (2013) 157–164.
[7] S. Luste, S. Vilhunen, S. Luostarinen, Effect of ultrasound and addition of bacterial product on hydrolysis of by-products from the meat-processing industry, Int. Biodeter. Biodegr., 65(2) (2011) 318–325.
[8] S.H. Joo, F.D. Monaco, E. Antmann, P. Chorath, Sustainable approaches for minimizing biosolids production and maximizing reuse options in sludge management: A review, J. Environ. Manage., 158 (2015) 133–145.
[9] V.K. Tyagi, S.-L. Lo, Sludge: A waste or renewable source for energy and resources recovery? Renew. Sust. Energ. Rev., 25 (2013) 708–728.
[10] E. Jardé, L. Mansuy, P. Faure, Organic markers in the lipidic fraction of sewage sludges, Water Res., 39 (2005) 1215–1232.
[11] M.N. Siddiquee, S. Rohani, Lipid extraction and biodiesel production from municipal sewage sludges: A review, Renew. Sust. Energ. Rev., 15 (2011) 1067–1072.
[12] E. Revellame, R. Hernandez, W. French, W. Holmes, E. Alley, Biodiesel from activated sludge through *in-situ* transesterification, J. Chem. Technol. Biot., 85 (2010) 614–620.
[13] N. Sangaletti-Gerhard, M. Cea V. Risco, R. Navia, *In-situ* biodiesel production from greasy sewage sludge using acid and enzymatic catalysts, Biores. Technol., 179 (2015) 63–70.
[14] A. Mondala, K. Liang, H. Toghiani, R. Hernandez, T. French, Biodiesel production by *in-situ* transesterification of municipal primary and secondary sludges, Biores. Technol., 100 (2009) 1203–1210.
[15] G. Pokoo-aikins, A. Heath, R.A. Mentzer, M.S. Mannan, W.J. Rogers, M.M. El-Halwagi, A multi-criteria approach to screening alternatives for converting sewage sludge to biodiesel, J. Loss Prevent. Proc., 23 (2010) 412–420.
[16] O.K. Choi, J.S. Song, D.K. Cha, J.W. Lee, Biodiesel production from wet municipal sludge: Evaluation of *in-situ* transesterification using xylene as a cosolvent, Biores. Technol., 166 (2014) 51–56.
[17] L.H. Huynh, P.L.T. Nguyen, Q.P. Ho, Y.-H. Ju, Catalyst-free fatty acid methyl ester production from wet activated sludge under sub critical water and methanol condition, Biores. Technol., 123 (2012) 112–116.
[18] J.M. Encinar, A. Pardal, G. Martinez, Transesterification of rapeseed oil in subcritical methanol conditions, Fuel Process Technol., 94 (2012) 40–46.
[19] P.J. Garcia-Moreno, M. Khanum, A. Guadix, E.M. Guadix, Optimization of biodiesel production from waste fish oil, Renew. Energ., 68 (2014) 618–624.
[20] F. Ma, M.A. Hanna, Biodiesel production: a review, Biores. Technol., 70(1) (1999) 1–15.
[21] Z. Ullah, M.A. Bustam, Z. Man, Biodiesel production from waste cooking oil by acidic ionic liquid as a catalyst, Renew. Energ., 77 (2015) 521–526.
[22] L. di Bitonto, A. Lopez, G. Mascolo, G. Mininni, C. Pastore, Efficient solvent-less separation of lipids from municipal wet sewage scum and their sustainable conversion into biodiesel, Renew. Energ., 90 (2016) 55–61.
[23] L.E. Rincón, J.J. Jaramillo, C.A. Cardona, Comparison of feedstocks and technologies for biodiesel production: An environmental and techno-economic evaluation, Renew. Energ., 69 (2014) 479–487.
[24] T.P. Hensarling, T.J. Jacks, Solvent extraction of lipids from soybeans with acidic hexane, J. Am. Oil Chem. Soc., 60 (1983) 783–784.
[25] P.L. Tran-Nguyen, A.W. Go, S. Ismadji, Y.-H. Ju, Transesterification of activated sludge in subcritical solvent mixture, Biores. Technol., 197 (2015) 30–36.
[26] E. Lotero, Y. Liu, D.E. Lopez, K. Suwannakarn, D.A. Bruce, J.G. Goodwin, Synthesis of biodiesel via acid catalysis, Ind. Eng. Chem. Res., 44 (2005) 5353–5363.
[27] M.K. Lam, K.T. Lee, A.R. Mohamed, Homogeneous, heterogeneous and enzymatic catalysis for transesterification of high free fatty acid oil (waste cooking oil) to biodiesel: A review, Biotechnol. Adv., 28(4) (2010) 500–518.
[28] S. Amit, Biodiesel: Production and Properties, The Royal Society of Chemistry, Cambridge, 2012.
[29] S. Dufreche, R. Hernandez, T. French, D. Sparks, M. Zappi, E. Alley, Extraction of lipids from municipal wastewater plant microorganisms for production of biodiesel, J. Am. Oil Chem. Soc., 84 (2007) 181–187.
[30] M. Olkiewicz, A. Fortuny, F. Stüber, A. Fabregat, J. Font, C. Bengoa, Evaluation of different sludges from WWTP as a potential source for biodiesel production, Procedia Eng., 42 (2012) 634–643.
[31] M. Kumar, P. Ghosh, K. Khosla, I.S. Thakur, Biodiesel production from municipal secondary sludge, Biores. Technol., 216 (2016) 165–171.
[32] D. Mara, N. Horan, Handbook of Water and Wastewater Microbiology, London: Academic Press, 2003.
[33] L. Shen, D.-K. Zhang, An experimental study of oil recovery from sewage sludge by low-temperature pyrolysis in a fluidised-bed, Fuel, 82 (2003) 465–472.
[34] A. Corma, G.W. Huber, L. Sauvanaud, O'Connor P, Biomass to chemicals: Catalytic conversion of glycerol/water mixtures into acrolein, reaction network, J. Catal., 257 (2008) 163–171.