Effective production of second generation bioethanol: Perspective study on wastewater pretreatment

インドネシアは世界最大のヤシ油生産国。その製造過程で出る有機廃棄物を利用し、食用資源と競合しないバイオ燃料を効率生産する方法を検証した。

Ajeng Arum Sari[1], Ahmad Faiz Ibadurrahman[2], Joko Waluyo[1], Muryanto[1], Feni Amriani[1], Dian Burhani[1], Yanni Sudiyani[1], Haznan Abimanyu[1]

[1] Researcher, Research Center for Chemistry, Indonesian Institute of Sciences, Indonesia

[2] Student, Department of Chemical and Biological Engineering, National Institute of Technology, Wakayama College, Japan

Abstract

Over the last ten years, there has been a dramatic increase in bioethanol production based on sugar cane and other vegetables. However, it faced a drawback as the high price of raw materials caused the production cost to be higher too. There is a need to explore alternative feedstocks such as lignocellulosic biomass because of its abundance in many countries but still neglected. Indonesia is the largest producer of palm oil in the world, and has high amount of oil palm empty fruit bunches (OPEFB) which could be utilized for bioethanol production. It consists of four main processes: pretreatment, hydrolysis, fermentation, and distillation of product ethanol. Pretreatment process used 600 kg OPEFB as a feed, and generated 76.46 kg bioethanol and 3,000 liters wastewater (called "black liquor") containing high COD and potentially toxic chlorinated compounds. Many researches regarding optimization process of bioethanol production and life cycle analysis of the process have been conducted. However, research concerning the wastewater pretreatment is still limited. Currently, we are developing technology for degradation of black liquor using combined treatment that consists of coagulation, Fenton, combination coagulation-Fenton. Each method has the ability to degrade black liquor in the range 50-98%. However, these systems also produce a sludge as new waste. Therefore, we are developing new technology to utilize this sludge as an environment-friendly activated carbon material based on Fe and Al. In our best knowledge, it is the first report regarding the utilization of black liquor sludge for activated carbon. This study was conducted considering the environmental aspect which should be applied through the development of an integrated engineering process for black liquor in production of the second generation bioethanol.

Keywords bioethanol, oil palm empty fruit bunches, wastewater treatments

Introduction

Bioethanol production based on sugar and starch has been getting familiar to substitute fossil fuels due to limited supplies and global warming issues. Unfortunately, this development has been retarded by the growing concerns of competition with food availability, actual net energy output, restrictions on land-use, and the high production cost[1]. On the other hand, lignocellulosic biomass from agricultural waste is available in abundance in many countries, but not well utilized. It does not need fertile land and can improve the emission balance of greenhouse gasses[2]. The bioethanol derived from non-edible lignocellulosic biomass is called the "second generation bioethanol."

This paper is a concise overview of the basic concepts in bioethanol production from oil palm empty fruit bunches. There are still some challenges which should be faced in wastewater treatment from pretreatment process of second-generation bioethanol. For this reason, three advantageous methods, regarding black liquor's efficient degradation, including PAC coagulation, Fenton, and Fenton-PAC were studied. At the end of this paper, the potential conversion of black liquor sludge to be activated carbon is also discussed.

Potential of agricultural wastes in Indonesia

In Indonesia, the commonly used agricultural residues for bioethanol production are mainly derived from oil palm(empty fruit bunches and fronds), rice (husk and straw), corn (corn cob), sugar cane (bagasse), and from forest-product waste[3]. Table 1 shows the summary for the potential of bioethanol production from agricultural wastes in Indonesia.

Bioconversion of oil palm empty fruit bunch to bioethanol

As the largest palm oil producer in the world, Indonesia produce crude palm oil which leaves oil palm empty fruit bunch (OPEFB) as a waste with comparison mass ratio 1:1.1[4]. As a lignocellulosic biomass, OPEFB contains cellulose (29.9-37.26%), hemicellulose (13.74-18.6%), and lignin (27.6-31.68%)[4,5,6]. Out of the three majority contents of lignocellulosic biomass only cellulose and hemicellulose can be converted to ethanol. Research Center for Chemistry, Indonesian Institute of Sciences has been developing the technological process for bioethanol production from OPEFB. The process itself consists of four steps: pretreatment to breakdown the main components, hydrolysis of cellulose to produce sugars, fermentation of sugars to ethanol, and distillation to obtain purified ethanol.

The primary purposes of pretreatment process are removing lignin and hemicellulose, increasing surface area and to fractionate amorphous cellulose[7,8,9,10]. The yield of cellulose hydrolysis is increased four-fold after pretreatment process[11]. There are several types of pretreatment process, including physical/ mechanical, chemical, biological, or a combination of these types[10,12,13,14,15,16]. The chemical pretreatment of lignocellulosic can use either dilute or strong acid, and sodium hydroxide (NaOH). For this study, we used alkaline pretreatment (NaOH). It was aimed to alter the structure of cellulosic biomass by removing lignin and hemicelluloses, so that the cellulose became more accessible to the enzymes that convert carbohydrate polymers into fermentable sugars[16]. The degree of polymerization and crystallinity will decrease which provokes lignin structure disruption[8]. During alkali pretreatment, lignin and hemicellulose are solubilized and/or decomposed in the aqueous phase result in a soluble fraction containing hemicelluloses and lignin degradation products, while cellulose remain in the solid fraction result in an insoluble cellulose-rich fraction[17,18,19].

However, the production of 76.46 kg bioethanol using 600 kg oil palm empty fruit bunches resulted in 3000 liters wastewater from alkaline pretreatment process. This wastewater is called "black liquor". It has black color with high COD (1,043 ppm) and also may consist potentially toxic chlorinated compounds, suspended solids, phenolics, and resin along with lignins.

Table 1. Production of agricultural wastes and bioethanol potential in Indonesia

Types	Year	Production (million tons) / year	Bioethanol potential (liter / ton)
Empty fruit bunches	2012	25.52	160.57
Palm oil fronds	2012	49	132.72
Rice straw	2012	0.02	99.43
Rice husks	2012	0.015	99.43
Corn cob	2012	1.94	0.13
Sugarcane	2011	0.3	75

Modified from Sudiyani et al. (2015)[3]

The second process is saccharification. The objective for this is to breakdown the pretreated cellulosic molecules into simple sugar by using enzymatic hydrolysis. Enzymatic hydrolysis is the key to cost-effective ethanol production[11]. The saccharification that we conducted in this study did not generate waste as it simultaneously carried out with the fermentation process.

The biomass is hydrolyzed by cellulolytic enzymes into fermentable sugars, which are directly fermented to ethanol. The main requirement for the microorganisms used in fermentation is it should utilize a broad range of substrates, high ethanol yield, titer and productivity, and high tolerance to ethanol, temperature, and inhibitors presence in hydrolysate[11]. The main advantages of simultaneous saccharification and fermentation process are comparatively lower costs, higher ethanol yields due to a removal of feedback inhibition on enzymatic saccharification[20]. Unfortunately, microorganisms for the different optimum conditions for enzyme hydrolysis in simultaneous saccharification and fermentation is rarely found[21]. Currently, we are developing the production of glutathione and yeast extracted from this wastewater.

Wastewater treatment in bioethanol production

Green product innovation is the interaction between technological innovation and sustainability that applied in industry. Wastewater treatment plays a vital role in people's daily lives regarding the purification of wastewater and the disposal of ready-for-reuse water to human society. For an industry, wastewater treatment is conducted to fulfill the obligations of environmental management and regulation and has become a branding as a green industry and product. The main focuses of the green industry are waste management, utilization of renewable energy as oil substitution, reduction of hazardous substances, and increase of environment-friendly materials with the minimum cost.

Considering how countries and international organizations compete to produce alternative energy, we tried to address this issue. The assessment of environmental and product dissemination as well as training, capacity building and monitoring which help achieving the sustainability criteria should be identified. Furthermore, planning and partnerships with public agencies and donors should also be integrated into the project design to support sustainability.

Unfortunately, waste issue in bioethanol production is often neglected. The United Nations declared the period of 2014 – 2024 to be the "Decade of Sustainable Energy for All", underlining the importance of energy access for sustainable development in developing countries. Therefore, to achieve a sustainable process, wastewater treatment should be integrated into designing the research for energy production. Hazardous waste generated from the bioethanol production should also be considered because it also poses a danger for environment sustainability. In this research, we integrated Indonesian clean energy technologies with waste management and life cycle assessment in second generation bioethanol production. By this, we reduced water pollution whose adverse impacts to human health and environment are expected to obtain.

Our research has successfully obtained ethanol with purity concentration of 99.5% (v/v) and production capacity of 10 litres per day. Many attempts have been mainly directed toward the improvement of the potential of lignocellulosic waste to produce bioethanol, however only a few studies have conducted researches regarding wastewater treatment. A great variety of physical, chemical, biological processes, and the combination of them, have been investigated for black liquor treatment in the paper manufacturing process. Even though black liquor only contributes 10-15% of total wastewater but it affected approximately 95% of the total pollution load of pulp and paper mill effluents[22]. Therefore, before released to the environment, the wastewater should be treated by removing or

Table 2. Wastewater treatment types and their unit operations

Methods	Unit operations
Physical	• Screening • Comminution • Flow equalization • Sedimentation • Flotation • Granular filtration
Chemical	• Chemical precipitation • Adsorption • Disinfection • Dechlorination • Coagulation • Advanced Oxidation Process (Fenton, photo-Fenton)
Biological	• Activated sludge • Aerated lagoon • Trickling filters • Rotating biological contactors

Modified from Zwain and Dahlan (2014)[22]

reducing certain harmful constituents using physical, chemical, and biological methods. Table 2 shows several methods for wastewater treatment and their processes.

Coagulation and Fenton methods are commonly used to treat organic pollutants including black liquor. Polyaluminium Chloride (PAC) has been found to be an effective coagulant to treat pulp and paper mill and dyes wastewater. It was able to reduce COD and color in pulp paper mill wastewater about 84 % and 92%, respectively, and precipitated about 99.5% suspended solid[23]. Fenton method has the ability to destruct the organic compound structure by using OH radical system[24]. Torrades et al.[25] reported that COD and color were removed 94% and 80%, respectively, by using this method.

Up to now, black liquor from the pretreatment process of bioethanol production in the pilot plant at Research Center for Chemistry, Indonesian Institute of Sciences, is discharged directly into the environment. It evaporates naturally with the hazardous compound still presence in this liquor. To minimize the amount of black liquor, we have reused black liquor in the pretreatment process[6,18]. However, black liquor treatment is still needed if black liquor will be discharged into the environment. Direct discharge of black liquor is prohibited by Indonesian Government Regulation No. 101 of 2014. Therefore, to fulfill this regulation, we have been developing black liquor wastewater treatment combining several technologies including coagulation using Poly Alumunium Chloride (PAC), Fenton method using $FeSO_4$-H_2O_2, and combination PAC-Fenton. Figure 1 provides the scheme for this research.

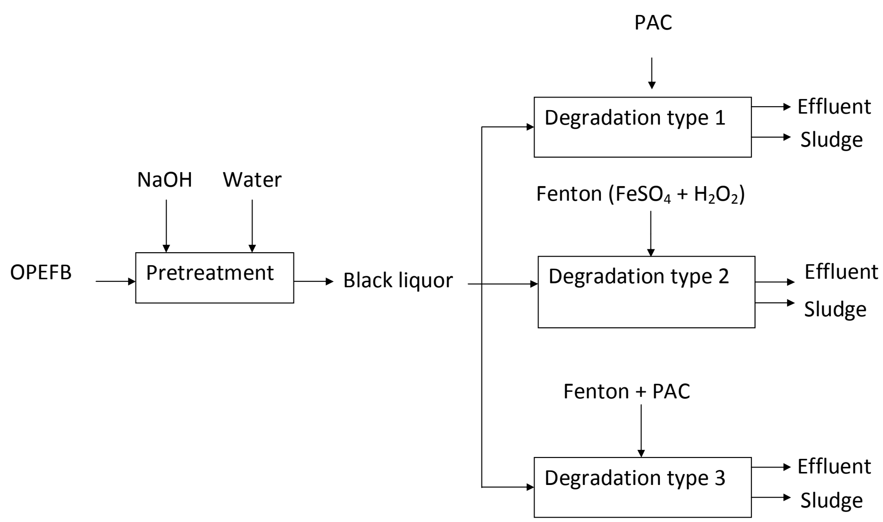

Figure 1. Research scheme of black liquor wastewater treatment

The coagulation and Fenton behavior's were evaluated regarding lignin removal, sludge weight, decolorization of black liquor, and COD. From all combination, the addition of PAC+Fenton gave the highest decolorization of black liquor and degradation of lignin. On the other hand, the maximum removal of COD and the lowest sludge weight were obtained from Fenton process. Fenton reagent is a reaction between hydroxyl radical and ion Fe^{2+}. Ion Fe^{2+} was commonly used as coagulant. The increasing of H_2O_2 also increase the black liquor decolorization due to the decomposition of $Fe^{2+}/^{3+}$ions with free hydroxyl radicals at a higher rate. The action of Fenton reagent depends on the H_2O_2 concentration because it can induce the molecular degradation.

Since the amount of Fenton reagent used is lower than the amount of coagulant, the sludge produced was also fewer. The addition of PAC increased the performance of Fenton reagent, as the PAC could coagulate organic compound and decrease the pH[26]. Acidic condition after PAC addition was suitable for the precipitation of lignin. Lignin fragments with average molecular weight ranging from 880-1,200 can be broken into small fragments by using coagulation and Fenton method. However, the remaining of turbidity caused by the presence of 5-7 phenolpropane units could not be efficiently removed only by coagulation method[27].

Study for impact assessment of wastewater treatment

Further data in Table 3 can be used to perform a life cycle analysis in a larger scale. Hence, the performance of these technologies should be tested first at pilot-scale, before ready to be applied in industrial scale. Before conducting the Life Cycle Assessment (LCA), the first step to do is to set the system boundary. Figure 1 ilustrated the system boundary for the black liquor treatment. In this study, the pretreatment process of bioethanol production is not included inside the boundaries. Land use is not taken into analysis because this research was carried out in laboratory-scale. Another data that should be considered is the energy inputs (electricity provided for instrumentation). SimaPro 7.3.3 was used to select impact categories, characterization models, and optional (normalization, grouping, and weighting) elements of the life cycle impact assessment (LCIA) according to ISO 14040.

Table 3. Degradation efficiency ofcoagulation and Fenton process at optimal operating parameters

Parameter	Coagulation (PAC)	Fenton (FeSO4-H2O2)	PAC + Fenton
Decolorization of black liquor (%)	70.64	51.45	97.82
Degradation of lignin (%)	68.28	24.85	98.91
Removal of COD (%)	19.46	54.75	45.25
Sludge weight (gram)	2.76	0.69	3.97

Using three types of wastewater treatment, several parameters in black liquor were successfully reduced. The optimum result from each treatments were showed in Table 3.

Conversion of black liquor sludge to activated carbon

The next process is to convert the sludge generated from the coagulation and Fenton process to be a value added product. The important factors which should be considered in making these advanced materials are efficiency, low cost, and will not generate any new waste (zero waste). One of the alternatives is to convert to activated carbon. To our knowledge, this is the first study regarding the utilization of bioethanol black liquor after coagulation process to be activated carbon. This research proposed an alternative technology for developing activated carbon from black liquor sludge that can meet environmental sustainability criteria and also can be applied in pulp industry. This project is technically feasible to implement because the abundant availability of resources including black liquor, coagulant and Fenton reagent. Activated carbon is a potential product which can be used directly and with appropriate maintenance, it could be used for long term. This technology establishes sustainable recycling process, consequently supported the environmental and practical value.

The generated dry sludge formed in prior process was subjected to carbonization-activation process which formed activated carbon. Carbonization is the pyrolysis process of raw material to remove non-carbonaceous elements. While, activation is a physical and chemical process that ensures the precursor has a porous structure so that it has a large surface area. This process enlarged micropores, which related to the development of the surface area which is necessary for adsorption.

In this experiment, the dry black liquor sludge was subjected to activation process with steam activation because using chemical activation could generate chemical waste which pose negative impact to the environment[28].

30 gram of activated carbon was obtained from 1 liter of black liquor sludge (Figure 2). The surface morphology of activated carbon from black liquor sludge was observed using SEM Hitachi SU300 at 1,00K x magnification. The SEM image of sample shows coarse surfaces, clumping, and some impurities. It was assumed that the impurities came from the presence of sodium in activated carbon. S_{BET} value for activated carbon from black liquor sludge was 164.45 m^2/g. This activated carbon was tested to use to adsorp methylene blue. Methylene blue is one of the most cationic dying materials for wood, silk, and cotton that commonly used for dye adsorption[29]. This dye can cause some harmful effects for water organisms and humans. In 0.5 g of PAC-based activated carbon, 100 ppm methylene blue was successfully decolorized up to 98% only for 30 min.

Lignocellulosic biomass has long been advocated as an essential feedstock for cost-effective bioethanol production in an environment-friendly and sustainable manner because of the abundance of lignocellulose-rich agricultural wastes/residues. Up to now research on utilization of agricultural residues for second-generation bioethanol production has shown very encouraging results worldwide. Our laboratory experiment and pilot-scale tests demonstrated promising result for bioethanol production from oil palm empty fruit bunches. There remains several challenges that should be overcome to make the process economically feasible, including: (1) handling of agricultural waste; (2) effective pretreatment technology; (3) effective cellulolytic enzymes; (4) use of the higher biomass loadings; (5) use of efficient fermentation process; (6) use of recombinant/metabolically engineered microbial strains; and finally (7) wastewater treatment.

2a 2b

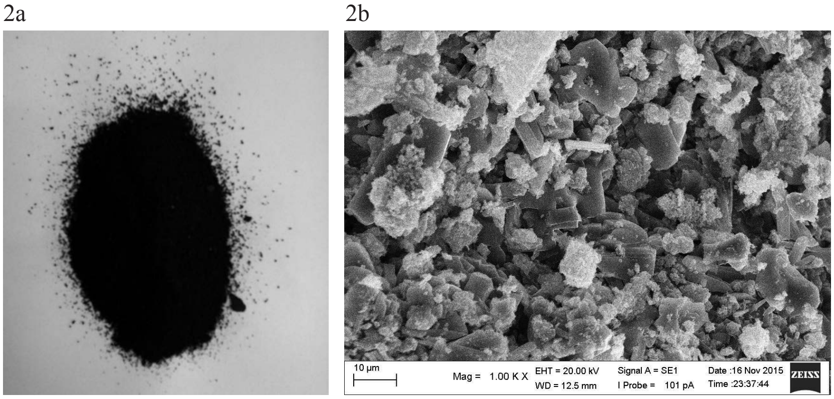

Figure 2. 2a: Appearance of activated carbon from black liquor sludge; 2b: SEM of activated carbon from black liquor sludge

When conducting research regarding bioethanol production which aims to be competitive and economically acceptable, the production cost should be considered[30]. The cost of feedstock and cellulolytic enzymes are the two critical parameters for low-cost ethanol production. The cost for biomass feedstock represents around 40 % of the ethanol production cost. The use of integrated approach which consists of an efficient pretreatment process, low cost enzyme, and super microbes for fermentation could also improve the ethanol production economically. The total cost of bioethanol production will be dropped to $0.2-0.5/l[31,32].

Conclusions

The utilization of agricultural wastes such as oil palm empty fruit bunches for bioethanol production is a cost-effective and environment-friendly approach to achieve sustainable process. Recent research progress in the fields of wastewater treatment to remove certain harmful constituents in bioethanol production from oil palm empty fruit bunches is indeed proved to be a feasible technology to support energy security in very near future. We have conducted Fenton and coagulation-flocculation process to treat black liquor wastewater obtained from the pre-treatment process of bioethanol production in Research Center for Chemistry-LIPI. Furthermore, we successfully developed the technology to generate an environment-friendly activated carbon material based on Al from black liquor sludge in bioethanol wastewater. This activated carbon was successfully decolorized methylene blue to a satisfactory result. This research resulted in an innovative product from black liquor sludge as an effort to fulfill a zero waste program. To our knowledge, activated carbon is still considered as a high-performance adsorbent which commonly used in wastewater treatment. So, this study was successfully contributed the environmental and practical value in the attempt to achieve a sustainable recycling process of wastewater from bioethanol production from oil palm empty fruit bunches in Indonesia.

Acknowledgments

This research was partly supported by Indonesia Science Toray Foundation Fiscal Year 2014 and INSINAS Ministry of Research, Technology and Higher Education Fiscal Year 2016. The authors are grateful for Ministry of Research, Technology, and Higher Education for financial support during participating the 3rd Asia Future Conference, Kitakyushu, Japan.

References

1) de Oliveira, M.E.D.; Vaughan, B.E.; Rykiel Jr, E.J. (2005) Ethanol as fuel: energy, carbon dioxide balances, and ecological footprint. BioScience, 55, 593-602
2) Wiloso, E.I.; Heijungs, R.; de Snoo, G.R. (2012) LCA of second generation bioethanol: A review and some issues to be resolved for good LCA practice. Renewable and Sustainable Energy Reviews, 16,7, 5295-5308
3) Sudiyani, Y.; Sembiring, K.C.; Barlianti, V.; Adilina, I.B. (2015)The opportunity of bioethanol production from lignocellulosic agricultural waste in Indonesia. In *Energy Science and Technology*. Studium Press LLC, 529-550.
4) Sudiyani, Y.; Styarini, D.; Triwahyuni, E.; Sudiyanmanto, Sembiring, K.C.; Aristiawan, Y.; Abimanyu, H.; Han, M.H. (2013) Utilization of biomass waste empty fruit bunch fiber of palm oil for bioethanol production using pilot-scale unit. Energy Procedia, 32, 31-38.
5) Sari A.A.; Kristiani, A.; Tachibana, S.; Sudiyani, Y.; Abimanyu, H. (2014) Mechanisms and optimization of oil palm empty fruit bunch as pre-grown source for white-rot fungus to degrade DDT. Journal of Environmental Chemical Engineering, 2, 1410-1415.
6) Muryanto, M.; Triwahyuni E.; Hendarsyah, H.; Abimanyu, H. (2015a). Reuse balck liquor of alkali pretreatment in bioethanol production. Energy Procedia, 68, 236-243.
7) Sun Y.; and Cheng, J. (2002) Hydrolysis of lignocellulosic materials for ethanol production : a review, Bioresources Technology, 83, 1-11.
8) Kumar, P.; Barrett D.M.; Delwiche M.J.; Stroeve P. (2009) Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. Ind Eng Chem Res, 48, 8, 3713-3729.
9) Cardoso,W.S.; Tardin F.D.; Tavares, G.P.; Queiroz P.V.; Mota S.S.; Catarina M.; Kazuya M.; Queiroz J.H.D. (2013) Use of sorghum straw (sorghum bicolor) for second generation ethanol production: pretreatment and enzymatic hydrolysis. Quim Nova, 36, 5, 623-627.

10) Kristiani, A.; Effendi N.; Styarini, D.; Aulia F; Sudiyani Y (2016) The effect of pretreatment by using electron beam irradiation on oil palm empty fruit bunch. Atom Indonesia, 42,1, 9-12.

11) Saini, J.L.; Saini, R.; Tewari, L. (2015) Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: concepts and recent developments. 3 Biotech, 5, DOI 10.1007/s13205-014-0246-5, 337-353

12) Mosier, N.; Wyman, C.; Dale B.; Elander, R.; Lee, Y.Y.; Holtzapple, M.; Ladisch, M. (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol 96, 673–686

13) Taherzadeh, M.J. and Karimi, K. (2008) Pretreatment of lignocellulosic wastes to improve ethanol and biogass production: A review. International Journal Molecular Sciences, 5, 337-353

14) Hu, G.; Heitmann, J. A.; and Rojas, O. J. (2008) Feedstock pretreatment strategies for producing ethanol from wood, bark and forrest residues. BioResources, 3, 270-294.

15) Hendriks, A. T. W. M.; and Zeeman, G. (2009). Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresource Technology, 100, 10-18.

16) Alvira, P.; Tomás-Pejó, E.; Ballesteros, M.; and Negro, M. J. (2010) Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. Bioresource Technology, 101, 4851-4861

17) Carvalheiro, F.; Duarte, L.C.; Girio,F.M. (2008) hemicellulose biorefineries: a review on biomass pretreatments. J.Sci Ind. Res, 67, 849-864.

18) Muryanto, M.; Triwahyuni, E.; Abimanyu, H.; Cahyono, A.; Cahyono, E. T., Sudiyani, Y. .(2015b). Alkaline Delignification of Oil Palm Empty Fruit Bunch using Black Liquor from Pretreatment. Procedia Chemistry, 16, 99-105.

19) Sudiyani,Y.; Triwahyuni,E.; Muryanto, M.; Dian Burhani, D.; Waluyo, J.; Anny Sulaswaty, A.; Abimanyu. H. (2016) Alkaline Pretreatment of Sweet Sorghum Bagasse for Bioethanol Production. Journal of Renewable Energy Development 5 (2) :113-118

20) Lin Y.; and Tanaka S. (2006). Ethanol fermentation from biomass resources: current state and prospects. Appl Microbiol Biotechnol. 627-642

21) Ballesteros, M.; Oliva, J. M.; Negro, M. J, Manzanares, P.; Ballesteros I. (2004) Ethanol from lignocellulosic materials by a simultaneous saccharification and fermentation process (SFS) with *Kluyveromyces marxianus* CECT 10875 . Process biochemistry 39: 1843-1848.

22) Zwain, H.M.; and Dahlan, I. (2014) Biological treatment of recycled paper mill wastewater using modified anaerobic inclining-baffled bioreactor (MAIB-R). In Wastewater Engineering Advanced Wastewater Treatment Systems. IJSR Publications, 72-86

23) Irfan, M.; Tahir, B.; Naz, I.; Naeem, A.; Ruaf, A.K.; Amir, S. (2013) The removal of COD, TSS, and colour of black liquor by coagulation-floculation process at optimized pH, settling and dosing rate. Arabian Journal Chemistry, doi:10.1016/j.arabjc.2013.08.007, 1878-5352

24) Mahmud, K.; Hossain, M.D.; Ahmed,S. (2011)Advanced landfill leachate treatment with least sludge production using modified Fenton process. International Journal of Environmental Sciences, 2, 259-270

25) Torades, F.; Saiz, S.; Garcia-Hortal, J.A (2011) Using central composite experimental design to optimize the degradation of black liquor by Fenton reagent. Desalination, 268, 97-102

26) Peavy, S.H.; Rowe, D.R.; Tchobanoglos, G. (1985) Environmental Engineering. Int. Ed. Mc-Graw Hill; New York

27) Rojas, O.J.; Song, J.; Argyropoulos, D.S. (2006) Lignin separation from kraft black liquors by tangential ultrafiltration. Science and Technology, 88, 88–95

28) Amriani, F.; Barlianti, V.; Muryanto, M.; Sari, A.A. (2015). Activated Carbon from Lignin Based Black Liquor Coagulated by Polyaluminium Chloride. Procedia Chemistry 16. 134-140.

29) Salleh, M.A.M.; Mahmoud, D.K.; Karim, W.A.W.A.; Idris, A. (2011) Cationic and anionic dye adsorption by agricultural solid wastes: a comprehensive review. Desalination, 280, 1-13

30) Subramanian, K.A.; Singal, S.K.; Saxena, M.; Singhal, S. (2005) Utilizationof liquid biofuels in automotive diesel engines: an Indian perspective. Biomass and Bioenergy, 29, 65–72

31) Wooley, R.; Ruth, M.; Sheehan, J.; Ibsen, K.; Majdeski, H.; Galvez, A. (1999) Lignocellulosic biomass to ethanol process design and economics utilizing co-current dilute acid prehydrolysis and enzymatic hydrolysis. In: Proceedings of current and futuristic scenarios. Technical report NREL/TP-580-26157. Golden, CO (USA), National Renewable Energy Laboratory, p 123

32) Aristidou, A.; and Penttila, M. (2000) Metabolic engineering applications to renewable resource utilization. Current Opinion Biotechnology, 11,187–198