Available online at www.sciencedirect.com

ScienceDirect

Energy Procedia 160 (2019) 670–676

Energy Procedia

www.elsevier.com/locate/procedia

2nd International Conference on Energy and Power, ICEP2018, 13-15 December 2018, Sydney, Australia

Enhancement of biogas generation in up-flow sludge blanket (UASB) bioreactor from palm oil mill effluent (POME)

MN Uddin[a], MA Rahman[a,*], J. Taweekun[c], K. Techato[a,d], M. Mofijur[b], M. Rasul[b]

[a]*Sustainable Energy Management, Faculty of Environmental Management, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand*
[b]*School of Engineering & Technology, Central Queensland University, Queensland 4701, Australia*
[c]*Department of Mechanical Engineering, Faculty of Engineering, Prince of Songkla University, Hatyai, Songkhla 90112, Thailand*
[d]*Municipal Solid Waste & Hazardous Waste Management Center of Excellence on Hazardous Substance Management, Bangkok 10330, Thailand*

Abstract

There are numerous different sorts of pre-treatment technique have been utilized with a few impediments regarding supportable natural administration in anaerobic assimilation for improvement of biogas generation. Albeit corrosive and salt pretreatment have a huge impact on the corruption of biomass, these techniques have some negative effects on the condition because of their perilous nature, while the enzymatic pre-treatment is more environmentally friendly. In this investigation is to streamline the biogas generation by enzymatic pre-treatment from Palm Oil Mill Effluent (POME) with assessing the improved biogas creation in a pilot scale bioreactor. It is to concentrate coordinate utilization of protein as enzymatic pre-treatment on POME to the improved generation of biogas. Proficiency of privately manufactured chemical with Up-flow Sludge Blanket (UASB) has not been researched in pilot scale previously. In this examination proficiency of COD expulsion and aggregate methane emanation is explored through pilot scale UASB bioreactor from POME through the application of enzyme and reviewed a study with the discussion.

Keywords: Biogas; Enzyme; Pre-treatment; Lignocellulose; Anaerobic digestion; Lipase; Cellulase.

1. Introduction

With the rapid increase of the world population, waste generation and demand for the natural resource are increasing with significant correlation as well as a reserve of fossil fuel is decreasing with alarming rate[1]. Biogas from lignocelluloses waste can be an alternative source of renewable energy. Palm oil industry is a prominent

* Corresponding author. Tel.: +61469851901.
E-mail address: m.rahman@cqu.edu.au

1876-6102
10.1016/j.egypro.2019.02.220

agricultural industry which has huge economic value in some tropical countries as in Thailand palm oil mill gains the fifth position. Thailand is the Third largest producer of palm oil in the world generates about 40 million tons of sludge palm oil (SPO) per year. Only 10% of palm oil fruit is used to produce oil and the rest 90% biomass can be used to produce renewable energy. Use of enzyme as a catalyst in pre-treatment for biogas production is regarded as an environmentally friendly technique. Lipases are industrially important enzymes[2] are capable to catalyze a vast range of reaction in nature [3, 4]. Lignocelluloses materials are resistant to degradation due to the rigid structure of cellulose, hemicelluloses, lignin [5]. Celluloses are able to hydrolyze cellulose into monomers which will lead faster anaerobic digestion to produce more biogas [6]. Therefore, this study focuses on the development of pretreatment process with lipase and cellulase enzymes for the monomers to enhance the biogas production from POME. The quantity of waste products in the world has been increasing considerably for many decades with the correlation between national gross domestic product (GDP) and waste generation per capita. Total worldwide estimation of waste production is incomplete and, in some cases, unreliable. A recent study suggests that the municipal solid waste alone generated globally exceeded 2 billion tons per year [7]. Biogas is regarded as an alternative energy source of fossil fuel. Waste from the agricultural industry can be a source of renewable energy. Agricultural plants residues are generated in huge quantity annually worldwide but are not utilized properly [8].

2. Anaerobic digestion

Anaerobic digestion may be defined as the decomposition of organic material in the absence of oxygen while simultaneously producing useful biogas consisting of mainly 60% methane (CH4), 35% carbon dioxide (CO2), and 5% ammonia (NH3) and other gases [9]. Anaerobic digestion is the most widely studied technology for organic treatments to alter the waste into renewable energy. Four different stages of reaction involved during the anaerobic digestion process: namely hydrolysis, acetogenesis, acetogenesis, and methanogens [10]. Through the process of anaerobic digestion, we can achieve energy recovery and pollution control. Many agricultural and industrial wastes are used for anaerobic digestion because they contain high levels of easily biodegradable materials [11].

Table 1. Characteristics of POME

Parameter (mg/L)a	[12]	MPOB (range)	MPOB mean	[13]	[14]	[15]	Discharge Standard, 1979
pH	4.5	3.4-5.2	4.2	4-5	4.7	4.8	5-9
BOD_3	25000	10250-43750	25000	25000-65714	25000	35432	50
COD	50000	15000-100000	51000	44300-102696	50000	79723	
TS	-	11500-79000	40000	40500-72058	40500	67200	
SS	19000	5000-54000	18000	18000-46011	18000	49300	100
VS		9000-72000	34000	34000-49300	34000	35935	
(O & G)	8000	130-18000	6000	4000-9341	4000-6000	17410	10
NH_3-N	35	4-80	35	35-103	35	173.8	150
TN	770	180-1400	750	750-770	750	873	200
P					180	277.7	
K					2270	5533	
Ca					439	607	
Mg					615	1065	
Fe					46.5	61	5.0
B					7.6		4.0
Zn					2.3	6.83	1.0
VFA						2287[b]	
Temp (˚C)	80-90						40

2.1. Alkaline pre-treatment /application of alkali

In alkaline pre-treatment bases, such as NaOH, $Ca(OH)_2$, KOH, and NH_3 H_2O, are used to remove lignin, hemicellulose, and/or cellulose, rendering lignocellulosic biomass more degradable to microbes and enzymes [16, 17]. Alkali pre-treatments studied for biogas production are summarized in Table 2.

Table 2. Effect of alkali pre-treatment on the lignocellulosic substrate to produce biogas [18]

Feedstock/	Optimum Pre-treatment condition	Results
Fallen leave	NaOH 3.5% and the substrate to inoculum (S/I) ratio 4.1	highest methane yield of 82 L/kg volatile solids (VS)
softwood spruce-hardwood birch	7.0% w/w NaOH solution at 100°C for 2 hr.	improved enzymatic hydrolyses of birch from 6.9% to 82.3% and spruce from 14.1% to 35.7%
Switch grass	29.5% reagent-grade aqueous ammonia at 5 L/ kg + cellulytic enzyme	0.15 10 0.36 m^3 CH_4 /kg VS
Corn Stover	solid-state anaerobic digestion with 5% NaOH	biogas yield of 372.4 L/kg VS (37% higher)
Sorghum straw	2% NaOH at 121°C for 1hr.	5.6 fold higher yield
Cotton Stalks	2% NaOH, 90 min, 121°C 15 psi, 2% NaOH 60 min, 121°C, 15 psi	60.8% cellulose conversion, Significant lignin degradation
Wheat straw	4.45% VS (44.5 g VS/L) of substrate + NaOH +37°C	353.2 L/kg VSa biogas, 87.5% higher than untreated substrate

2.2. Acid pre-treatment / application of acid

In Acid pre-treatment both inorganic and organic acids, including sulfuric acid (H_2SO_4), nitric acid (HNO_3), hydrochloric acid (HCl), phosphoric acid (H_3PO_4), acetic acid, and maleic acid, have been used for dilute acid pre-treatment, while H_2SO_4 has gained the most importance. Acid pre-treatments studied for biogas production are summarized in Table 3.

Table 3. Effect of acid pre-treatment on the lignocellulosic substrate to produce biogas

Feedstock	**Optimum pre-treatment condition**	**Results**	**References**
Sunflower	4% (w/w) HCl at 170°C in 1h	removed more than 90% of hemicelluloses	[19]
Sunflower oil cake (SOC)	Combined diluted acid at 170°C	highest yield (292-312) mL CH4/gVS	[20]
Sugarcane bagasse (SCB).	2% sulphuric acid 121°C 18 day, enzymatic hydrolysis	methane yield of 200 NL/ kg VS (15+/6% higher)	[21]
Cassava residue	157.84°C, 2.99% (w/w TS) H_2SO_4 for 20.15 min	methane yield (248 mL/g VS) was 56.96% higher	[22]
Herbal extraction residue	HPR was hydrolysed by MAHP for 30 min at a 1.2% (w/w).H_2SO_4	1272 mL after 19 days	[23]

2.3. Enzymatic pre-treatment / Enzyme application

Many researchers used enzyme as a co-digestion with chemical or physical treatment. As chemical treatment has some negative impact of on the environment, researchers are searching alternative way. Enzymatic pre-treatment can be one of the solutions. Enzymatic pre-treatments studied for biogas production are summarized in Table 4.

Table 4. Effect of Enzymatic pre-treatment on lignocelluloses substrate to produce biogas

Feedstock	**Optimum Pre-treatment condition**	**Results**	**References**
Pulp and paper sludge	*Saccharomyces cerevisiae* CICC 1001; batch reactor, pH 6.0 [24] total solids 6% enzyme loading 40 A.U./g VS	42.5 g/L ethanol (highest yield)	[25]
Wheat grass	In 1ststageNovozyme 342 (N342) prior AD and in 2nd stage (85% Celluclast (C15L) 1.5L + 15% (N188)) pH 7.6, 11-13 days	Biogas yield (L/g VS) (0.44 ± 4%) higher than that of without enzymatic pretreatment	[26]
Sewage sludge	A mixture of α-amylase and protease at the ratio of 3 at 50°C	The increase of hydrolysis rate from 10% to 68.43%	[27]

Sugar beet pulp & spent hops	Digestion with mix enzyme, 24 h hydrolysis	19% increase of biogas from sugar beet & 13% increase from spent hops	[28]
Switchgrass	The only use of pectinase, no chemical pre-treatment	Yields of 287.4 and 239.5 ml/g VS with pectate-lyase and poly-galacturonate respectively	[29]

2.4. Role of Cellulose and Lipase enzyme

Celluloses enzyme can breaks down cellulose, the main component of the cell walls of plant biomass. Sugar is a product of hydrolysis of cellulose is carried out by the aid of this enzymatic system. Major drawbacks to exploiting the commercial potential of cellulose are yield, stability, production costs and specificity[30]. Lipases are one of the important classes of an enzyme play a very important role as catalysts in the degradation of fat and food, in biotechnology, for the manufacturing of oleochemicals and for organic synthesis. The broad application is studied to a wide range of the substrate acceptance and stability in organic solvents which can be applied in hydrolysis reactions as well as ester synthesis [31].

3. Process

Diluted sugar solution (1.5 ml) was taken in a 25 ml test tube, 3 ml of DNS reagent was added and placed in a boiling water bath for 5 minutes. The tubes must be cooled immediately after boiling; 10 ml of distilled water have to be added and mixed thoroughly. The absorbance of the developed colour was measured at 540 nm. The absorbance values (after subtraction of the reagent blank) were then translated into glucose equivalent using a standard graph obtained by plotting glucose concentration (0.1-1.0 g/l) against absorbance. The colour develops only under alkaline conditions: was added 2 drops of 0.1 M NaOH for acidic samples. Determination of optimum mixed enzyme activity in enzymatic hydrolysis- Mixture of Lipase and Cellulose in different ratio was added with POME prior to anaerobic digestion. After 24 hours FFA and sugar content was measured. The ratio of lipase and cellulose will be1:1, 1:2, 1:3, and 1:4.

3.1. Determination of the efficiency of COD removal and VSS reduction with different OLR and enzyme dose

In this stage efficiency of COD removal has been measured by applying different enzyme dose and organic loading rate (OLR) through FCCCD. Three parameters will be manipulated in order to investigate the effects of them on the amount of COD removal and biogas production. Based on these parameters, the experiment was run for 20 runs. Design of 3 factors with 1 response has been shown in Figure 1.

Std	Run	Block	Factor 1 A:Lipase U/ml	Factor 2 B:Cellulase FPU/ml	Factor 3 C:OLR mg/L	Response 1 COD removal %
2	1	Block 1	1.00	-1.00	-1.00	
18	2	Block 1	0.00	0.00	0.00	
10	3	Block 1	1.00	0.00	0.00	
3	4	Block 1	-1.00	1.00	-1.00	
13	5	Block 1	0.00	0.00	-1.00	
5	6	Block 1	-1.00	-1.00	1.00	
14	7	Block 1	0.00	0.00	1.00	
19	8	Block 1	0.00	0.00	0.00	
9	9	Block 1	-1.00	0.00	0.00	
7	10	Block 1	-1.00	1.00	1.00	
16	11	Block 1	0.00	0.00	0.00	
4	12	Block 1	1.00	1.00	-1.00	
17	13	Block 1	0.00	0.00	0.00	
6	14	Block 1	1.00	-1.00	1.00	
8	15	Block 1	1.00	1.00	1.00	
12	16	Block 1	0.00	1.00	0.00	
1	17	Block 1	-1.00	-1.00	-1.00	
15	18	Block 1	0.00	0.00	0.00	
20	19	Block 1	0.00	0.00	0.00	
11	20	Block 1	0.00	-1.00	0.00	

Figure 1. General design with design expert software with 3 factor and 1 response.

3.2. Sub-culturing, inoculum preparation and SSB

The microbial strain, Candida cylindrical (ATCC 14830) used in this study was purchased from the American Type Tissue Culture, USA. C. cylindrical was grown on PDA plates at 28ºC for four days in an incubator (Incucell, Germany) and sub-cultured every two weeks. Each plate was washed with 10.0 ml sterile distilled water and the suspension was used to prepare the inoculum in the appropriate medium. Locally fabricated PKC Lipase through the solid-state fermentation (SSB) was produced according to the method suggested by [32, 33]. Trichoderma reesei RUTC-30 (ATCC 56765) was cultured on the PDA plate as inoculate source and incubated at 30°C for 8-10 days until the good sporulation was observed. Cellulose enzyme was produced and assayed through the method described by [34].

3.3. Measurement of Effect of PKC lipase and Cellulose enzyme on POME

Ensuring anaerobic condition 100 ml shake flask was put on a shaker for 24 hours at 150 rpm speed with different doses of PKC lipase with 5 g raw POME while the control sample was left without enzyme loading. Free fatty acids (FFA) content was measured titrating against sodium hydroxide (0.1N) with phenolphthalein indicator (Table 5). A set of the sterilized sample was put on shaker as the same process shown by [33, 35]. Ensuring anaerobic condition 100 ml shake flask was used at 120 rpm speed with different doses of cellulose enzyme with centrifuged 10g of raw POME while the control sample was left without enzyme loading and reducing sugar was measured after 12 hours and 24 hours according to the method suggested by [34]. A set of the sterilized sample was used as the same process. Effects of cellulose enzyme on POME have been shown in Figure 3.

4. Results and Discussion

Up-flow Sludge Blanket (UASB) is a suitable bioreactor to treat liquid waste through anaerobic digestion to produce biogas. 96% COD removal of POME was found by using UASB bioreactor. Data was collected and presented for analysis and discussions accordingly. With sterilized POME at pH range, 3.7 FFA (%) is higher than all other process condition. The reason behind higher FFA is there was no microbial effect on POME in sterilized condition while without sterilized condition may have a microbial effect on FFA content. [33] reported optimal activity of PKC lipase at pH range from 7 -8, at 25 -45˚C temperature while at pH lower than 4 range activity decreases 50% and above 60˚C no activity has been found.

Table 5. Effect of Lipase on POME at different condition.

Effect of lipase on POME												
	Without Sterilized						***Sterilized***					
The dose of Lipase (U/ml)	***pH 3.7***		***pH 4.5***		***pH 7***		***pH 3.7***		***4.5***		***pH 7***	
	FFA (%)	***Increase FFA (%)***	***FFA %***	***Increase FFA (%)***	***FFA %***	***Increase FFA (%)***	***FFA (%)***	***Increase FFA (%)***	***FFA (%)***	***Increase FFA (%)***	***FFA (%)***	***Increase FFA (%)***
Control	0.733		0.846		0.169		3.835		3.2712		3.2712	
2.468	0.790	7.72	1.015	20.00	0.225	33.33	3.948	+2.9465	3.4968	6.8966	3.4968	37.5
4.937	0.818	11.57	1.128	33.33	0.254	50	3.948	+2.9465	3.6096	10.344	3.6096	62.5
7.406	0.846	15.42	1.241	46.67	0.254	50	4.173	+8.8292	3.7224	13.793	3.7224	62.5
9.875	0.846	15.42	1.354	60.00	0.225	33.33	4.286	+11.603	3.8352	12.741	3.8352	50.0
12.343	0.790	7.72	1.410	66.67	0.225	33.33	4.286	+11.603	3.9480	20.689	3.9480	50.0

NaOH was added to increase pH level from 3.7 to 7, which itself neutralize the FFA. That's why we got lower FFA at the pH range 7 than at the pH range 3.7. About 66.67% more reducing sugar was obtained than control sample by

using 12.343 U/ml lipase dose at the pH range 4.5 with raw POME without sterilization. Using 9.875 U/ml lipase dose at the pH range 3.7 with sterilized, without sterilized condition 11.603 % and 15.42% higher FFA was found respectively than control sample while 62.5% higher FFA was found at pH range 7 with sterilized POME (Table 6).

Using 0.168 FPU/ml cellulose enzyme loading with sterilized POME at pH range 3.7, after 24 hours 34.67% higher reducing sugar was found that the control sample. Amount of reducing sugar has been found more after 24 hours than 12 hours on a shaker in both process conditions.

5. Conclusion

Sustainable management is a serious issue of the present world. To achieve a sustainable environment and to minimize the volume of organic waste, enzymatic pre-treatment could be an alternative over acid/base pretreatment processes for industrial applications. Although this is a preliminary stage of research, several studies show positive findings on enzymatic pretreatment method towards enhanced biogas production. Extensive researches are needed for future development. Pilot scale production of biogas in UASB bioreactor from POME through AD can be a milestone for future development. Cellulose and lipase have a significant effect on the POME to produce monomers towards more yield of biogas. To get the optimum condition to produce the highest range of biogas from POME by using locally produced enzymes more work needs to be done.

References

[1] M. Uddin, K. Techato, J. Taweekun, M. Rahman, M. Rasul, T. Mahlia, S. Ashrafur, An Overview of Recent Developments in Biomass Pyrolysis Technologies, Energies, 11 (2018) 3115.
[2] F. Hasan, A.A. Shah, A. Hameed, Industrial applications of microbial lipases, Enzyme and Microbial technology, 39 (2006) 235-251.
[3] A.S. de Miranda, L.S.M. Miranda, R.O.M.A. de Souza, Lipases: Valuable catalysts for dynamic kinetic resolutions, Biotechnology Advances, 33 (2015) 372-393.
[4] J.G. Duarte, L.L.S. Silva, D.M.G. Freire, M.C. Cammarota, M.L.E. Gutarra, Enzymatic hydrolysis and anaerobic biological treatment of fish industry effluent: Evaluation of the mesophilic and thermophilic conditions, Renewable Energy, 83 (2015) 455-462.
[5] E. Palmqvist, B. Hahn-Hägerdal, Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition, Bioresource technology, 74 (2000) 25-33.
[6] A.A. Klyosov, Trends in biochemistry and enzymology of cellulose degradation, Biochemistry, 29 (1990) 10577-10585.
[7] L. Giusti, A review of waste management practices and their impact on human health, Waste Management, 29 (2009) 2227-2239.
[8] M.F. Demirbas, M. Balat, Recent advances on the production and utilization trends of bio-fuels: A global perspective, Energy Conversion and Management, 47 (2006) 2371-2381.
[9] E. Bruni, A.P. Jensen, I. Angelidaki, Comparative study of mechanical, hydrothermal, chemical and enzymatic treatments of digested biofibers to improve biogas production, Bioresource technology, 101 (2010) 8713-8717.
[10] Y. Li, S.Y. Park, J. Zhu, Solid-state anaerobic digestion for methane production from organic waste, Renewable and Sustainable Energy Reviews, 15 (2011) 821-826.
[11] Y. Chen, J.J. Cheng, K.S. Creamer, Inhibition of anaerobic digestion process: a review, Bioresource technology, 99 (2008) 4044-4064.
[12] A. Ma, A.S. Ong, Treatment of palm oil steriliser condensate by an anaerobic process, Biological wastes, 23 (1988) 85-97.
[13] M.J. Chin, P.E. Poh, B.T. Tey, E.S. Chan, K.L. Chin, Biogas from palm oil mill effluent (POME): Opportunities and challenges from Malaysia's perspective, Renewable and Sustainable Energy Reviews, 26 (2013) 717-726.
[14] A. Ahmad, S. Sumathi, B. Hameed, Residual oil and suspended solid removal using natural adsorbents chitosan, bentonite and activated carbon: A comparative study, Chemical Engineering Journal, 108 (2005) 179-185.
[15] Y. ZHANG, Y. Li, C. Lina, L. Xiuhua, M. Zhijian, Z. ZHANG, Startup and operation of anaerobic EGSB reactor treating palm oil mill effluent, Journal of Environmental Sciences, 20 (2008) 658-663.
[16] Y. Sun, J. Cheng, Hydrolysis of lignocellulosic materials for ethanol production: a review, Bioresource Technol, 83 (2002) 1-11.
[17] M.J. Taherzadeh, K.J.I.j.o.m.s. Karimi, Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review, 9 (2008) 1621-1651.
[18] L.N. Liew, J. Shi, Y. Li, Enhancing the solid-state anaerobic digestion of fallen leaves through simultaneous alkaline treatment, Bioresource Technol, 102 (2011) 8828-8834.
[19] F. Monlau, A. Barakat, J.P. Steyer, H. Carrere, Comparison of seven types of thermo-chemical pretreatments on the structural features and anaerobic digestion of sunflower stalks, Bioresource Technol, 120 (2012) 241-247.
[20] F. Monlau, E. Latrille, A.C. Da Costa, J.P. Steyer, H. Carrere, Enhancement of methane production from sunflower oil cakes by dilute acid pretreatment, Appl Energ, 102 (2013) 1105-1113.
[21] M. Badshah, D.M. Lam, J. Liu, B. Mattiasson, Use of an Automatic Methane Potential Test System for evaluating the biomethane potential of sugarcane bagasse after different treatments, Bioresource Technol, 114 (2012) 262-269.
[22] Q.H. Zhang, L. Tang, J.H. Zhang, Z.G. Mao, L. Jiang, Optimization of thermal-dilute sulfuric acid pretreatment for enhancement of methane production from cassava residues, Bioresource Technol, 102 (2011) 3958-3965.
[23] C.Z. Liu, X.Y. Cheng, Microwave-Assisted Acid Pretreatment for Enhancing Biogas Production from Herbal-Extraction Process Residue, Energ Fuel, 23 (2009) 6152-6155.
[24] T. Yoshizaki, Y. Shirai, M.A. Hassan, A.S. Baharuddin, N.M. Raja Abdullah, A. Sulaiman, Z. Busu, Improved economic viability of

integrated biogas energy and compost production for sustainable palm oil mill management, Journal of Cleaner Production, 44 (2013) 1-7.
[25] Y. Lin, D. Wang, T. Wang, Ethanol production from pulp & paper sludge and monosodium glutamate waste liquor by simultaneous saccharification and fermentation in batch condition, Chem Eng J, 191 (2012) 31-37.
[26] R.T. Romano, R. Zhang, S. Teter, J.A. McGarvey, The effect of enzyme addition on anaerobic digestion of Jose Tall Wheat Grass, Bioresource Technol, 100 (2009) 4564-4571.
[27] Q. Yang, K. Luo, X.-m. Li, D.-b. Wang, W. Zheng, G.-m. Zeng, J.-j. Liu, Enhanced efficiency of biological excess sludge hydrolysis under anaerobic digestion by additional enzymes, Bioresource Technol, 101 (2010) 2924-2930.
[28] K. Ziemiński, I. Romanowska, M. Kowalska, Enzymatic pretreatment of lignocellulosic wastes to improve biogas production, Waste Management, 32 (2012) 1131-1137.
[29] J.-C. Frigon, P. Mehta, S.R. Guiot, Impact of mechanical, chemical and enzymatic pre-treatments on the methane yield from the anaerobic digestion of switchgrass, Biomass and Bioenergy, 36 (2012) 1-11.
[30] M.Z. Alam, A.A. Mamun, I.Y. Qudsieh, S.A. Muyibi, H.M. Salleh, N.M. Omar, Solid state bioconversion of oil palm empty fruit bunches for cellulase enzyme production using a rotary drum bioreactor, Biochemical Engineering Journal, 46 (2009) 61-64.
[31] J.Y. Chen, C.M. Wen, T.L. Chen, Effect of oxygen transfer on lipase production by Acinetobacter radioresistens, Biotechnology and bioengineering, 62 (1999) 311-316.
[32] A. Salihu, M.Z. Alam, M.I. AbdulKarim, H.M. Salleh, Optimization of lipase production by Candida cylindracea in palm oil mill effluent based medium using statistical experimental design, Journal of Molecular Catalysis B: Enzymatic, 69 (2011) 66-73.
[33] A.A. Elgharbawy, M.Z. Alam, H.M. Salleh, CHARACTERIZATION OF LOW-COST LIPASE BY SOLID-STATE FERMENTATION OF PALM KERNEL CAKE USING CANDIDA CYLINDRACEA, (2006).
[34] S.S. Rashid, M. Alam, M.I. Abdulkarim, H.M. Salleh, Optimization of the nutrient supplements for cellulase production with the basal medium palm oil mill effluent, (2009).
[35] T. Abbasi, S. Tauseef, S. Abbasi, Biogas and Biogas Energy: an introduction, in: Biogas Energy, Springer, 2012, pp. 1-10.