Journal of Energy and Safety Technology

RESEARCH ARTICLE Open Access

The Utilization of Pineapples Waste Enzyme for the Improvement of Hydrolysis Solubility in Aquaculture Sludge

M. Fatma Syahirah, R. Nazaitulshila*

School of Ocean Engineering, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

Corresponding author: nazaitulshila@umt.edu.my

Article History

Received: May 7, 2018 Received in revised form: November 2, 2018 Accepted: November 17, 2018 Published Online: February 10, 2019

Abstract

High volumes of sludge discharge from the aquaculture industry have relatively high pollutant content that may lead to severe local environmental problems. Anaerobic digestion is one of the technologies for sludge treatment that might be an efficient method to reduce salty aquaculture sludge production load. However, hydrolysis solubility is becoming a limitation step during the anaerobic digestion process when the occurrence of intermediate accumulation resulted from the conversion of non-soluble biopolymers to soluble organic compounds. Thus, pretreatment is required to increase the solubilization of pollutant parameters from aquaculture sludge before it is further treated in the anaerobic treatment. Therefore, this study focuses on the production of biocatalytic enzyme from the fermentation of pre-consumer supermarket waste such as pineapple dregs to increase the solubility of aquaculture sludge. The fermentation of the pineapples waste was produced via a three-month fermentation of the mixture of molasses, pineapple dregs and water, with the ratio of 1:3:10 in a tight plastic container. Apart from that, analyses showed that the enzyme possessed lipase, amylase and protease activity. The sludge solubilization pretreatment was performed at different pH values, with treatment time for 120 hours and the solubilization was evaluated by determination of Chemical Oxygen Demand (COD) and Total Ammonia Nitrogen (TAN) solubilization; the solubilization of COD and TAN increased by 80% and 50%, respectively. This finding showed that the pineapple enzyme has the capability to solubilize organic compound, which has the potential to improve hydrolysis in further anaerobic digestion process.

Keywords: Biocatalytic; enzyme; pineapple waste; hydrolysis solubility; aquaculture sludge

1.0 INTRODUCTION

In Malaysia, fish food is a main source of protein, subsequently, increasing the fish industry demand. Fisheries, or aquaculture sector, provide food security to the population in Malaysia. The National Agro-food Policy (2011–2020) reported that the annual demand of the sectors from year 2011 to 2020 would increase from 1.7 to 1.93 million tons [1]. Rapid growth in the sector activities had subsequently increased the effluents that effect the environment due to the use of high technology to maintain the production of fish or shrimp farming industries. This resulted in accumulated high pollutants and organic content discharges from the activities known as aquaculture sludge. The aquaculture sludge is mainly from the downstream activities such as fish feed, fish seed production and fish faeces. Typically, the large amount of aquaculture sludge discharged and pumped out from the pond bottom drain is characterized by the increased of ammonia, nitrites, nitrates, organic carbon, phosphates, suspended solids, and Chemical Oxygen Demand (COD) [2]. Thus, before disposing the sludge for further utilization such as landfilling, it has to be treated efficiently. The improper disposal of the salty aquaculture sludge to landfills is not a good practice because it can contaminate the surface and groundwater, thus causing environmental problems.

Anaerobic digestion is a technology approach to treating the aquaculture sludge that promotes clean technology, but also offers valuable byproducts. The four reactions that occur during the entire process of anaerobic digestion to form methane are hydrolysis, acidogenesis, acetogenesis, and methanogenesis stages. All these four reactions occur simultaneously and are independent of each other. However, there is a limitation step during the hydrolysis stage that may intermediate in the conversion of non-soluble biopolymers to soluble organic compounds in the digestion. Various pretreatment technologies have been developed in recent years to improve the sludge hydrolysis solubility such as chemical, thermal and biological pretreatments. Chemical and thermal pretreatments require high operating and handling costs. According to Im et al. [3], the biological method is preferred because it is considered as an environmentally-safe treatment. In addition, the presence of specific exoenzyme added to a system and several enzymatic pretreatments were believed to ensure the hydrolysis and degradation steps [4]. The biological method by enzymatic pretreatment has been reported to be an effective pretreatment to reduce the limitation in hydrolysis solubility and enhance the efficiency of anaerobic digestion of dairy wastewater, waste activated sludge, slaughterhouse wastewater, and sewage sludge [5-8]. Basically, the aquaculture sludge has the potential to be a resource recovery in anaerobic treatment, as well as reported from previous studies because the concepts and issues are similar. Nevertheless, there was a lack reported on the solubilization of the enzymatic pretreatment on aquaculture sludge.

To achieve a high degree of degradation, most studies done on the use of commercial hydrolytic enzyme were found to be high in cost and complicated, where some chemicals needed to be added for pretreatment which may lead to environment pollution. Thus, in this study, the pineapples waste, also known as pineapple enzyme, was preferred due to it being low cost, abundantly available and environmentally friendly, instead of using the commercial enzyme. This enzyme has an important role to achieve a high degree of degradation and act as a catalyst to speed up the reaction, similar to the commercial enzyme. Furthermore, the enzyme not only acts as additives to treat specific pollutants in wastewater, but also reduces the disposal of solid waste generated. Previous study has developed the enzyme bioproduct solution by fermentation of using organic solid waste, molasses and water, namely, the garbage enzyme [9]. Several studies have discovered the use of the enzyme bioproduct from organic waste to treat the synthetic grey water and domestic wastewater to reduce the pollutant content, and higher removals of ammonia nitrogen and phosphate were obtained from their findings [10,11].

In this study, the utilization of the pineapples waste as enzyme was studied. The biocatalytic of the enzyme was determined by identifying the biocatalytic properties at different conditions of the enzyme's pH. This study will focus on the potential of the enzyme produced from the pineapple waste as enzymatic pretreatment in hydrolysis solubility of aquaculture sludge.

2.0 METHODOLOGY

This section discusses the methodology and materials selection that were applied throughout this experiment or study.

2.1 Production and Characterization of Pineapple Enzyme

The pineapples waste was collected from local supermarket residues near the Kuala Terengganu area. The pineapples waste was put in a 2-liter airtight container along with the molasses and water. To produce the pineapple enzyme solution, the molasses, pineapples waste and water were mixed with a ratio 1:3:10, respectively, for period of three months for the fermentation process. The airtight container was labelled as pineapple enzyme and placed in a cool and dry area at room temperature. The gas formation during the fermentation was released daily for the first month and for the next two months, the gas released was once a month. After three months of fermentation, the solution of the enzyme was filtered and centrifuged at 3000 rpm for 30 minutes and stored at 4℃ prior to its use. The characterizations of the pineapple enzyme were conducted based on parameters such as pH, total solid (TS), volatile solid (VS), total suspended solid (TSS), volatile suspended solid (VSS), COD, and Total Ammonia Nitrogen (TAN) as a standard procedure [12].

2.2 Biocatalytic Activity of Pineapple Enzyme

In this present study, the biocatalytic activities of enzyme lipase, amylase and protease at two conditions of pH (3.5 and 7.0) were determined. This is due to presence of high amount of carbohydrates, proteins and lipids in the aquaculture sludge, which can be degraded by enzyme lipase, amylase and protease, respectively.

2.2.1 Lipase Activity

The pineapple enzyme solution was prepared triplicate and adjusted to the pH of 3.5 and 7.0 with added sodium citrate buffer and phosphate buffer, respectively. The lipase activity was determined by using the method by Pandey with a few modifications, where the mixture contains 0.05 ml of pineapple enzyme and 0.95 ml of substrate (1:9 of 3.0Mm p-NPPin 2 propanol, 0.4% Triton X100 and 0.1% Arabic gum) [13]. Then, it was incubated at 37℃ for 20 minute and read using the UV/VIS spectrophotometer with absorbance at 440 nm. The lipase activity was expressed as the amount of release of 1 mole of p-nitrophenol per minute of tyrosine equivalent per minute.

2.2.2 Amylase Activity

The pH of pineapple enzyme was adjusted at two conditions of pH, which are 3.5 and 7.0, by adding the sodium citrate buffer and phosphate buffer, respectively. The enzymatic assays were run triplicate. Amylase activity was measured by adapting the procedure used by Benfeld with little modifications to it [14]. The mixture of 0.50 ml of pineapples enzyme and 0.50 ml of 1.0% of starch solution was incubated at 25℃ for 10 minutes. Then, 1 ml of dinitrosalicylic acid color reagent was added. The mixture is then incubated in a boiling water bath for 5 minutes to allow reaction, and to cool to room temperature; the absorbance was read at a wavelength of 540 nm.

2.2.3 Protease Activity

The pineapple enzyme was adjusted into preferable pH, which are 3.5 and 7.0. Then, the sodium citrate buffer and phosphate buffer were adjusted until they reached the pH of 3.5 and 7.0, respectively. The protease assays were run in triplicate. The protease activity was analyzed by mixing 1 ml of pineapple enzyme with 1 ml of 2% of casein solution and pre-warmed for 10 minutes to allow reaction. Then, about 2 ml of trichloroacetic acid solution was added and incubated at 35℃ for 10 minutes. The mixtures were centrifuged at 3000 rpm. Then, about 1 ml of the clear supernatant was collected after the centrifugation of the mixtures, and then dissolved with 5 ml of sodium carbonate (Na_2CO_3) solution and 1 ml of Follin phenol reagent, following the procedure used by Lowry [15]. The mixtures were prepared and the absorbance value was measured at a wavelength of 660 nm using the UV/VIS spectrophotometer. The specific activity of protease was expressed as the amount of enzyme that releases 1 mg of protein by using casein as substrate equivalent per minutes.

2.3 Sampling of Aquaculture Sludge

The aquaculture sludge was collected from the pond bottom of a shrimp hatchery located in Setiu, Terengganu, and kept for 4℃. The characteristics of the aquaculture sludge were analyzed according to the standard method, namely, pH, TS, VS, TSS, TDS, VSS, COD, and TAN as shown in Table 1.

Table 1. Characteristic of Aquaculture sludge

Parameter	Units	Value
pH	-	6.50 ± 0.2^a
Total Solid (TS)	%	5 ± 0.1^a
Volatile Solid (VS)	%	39 ± 0.2^a
Total Suspended Solid (TSS)	mg/l	$57{,}450 \pm 17.0^a$
Total Disolved Solid (TDS)	mg/l	$705{,}717 \pm 10.4^a$
Volatile Suspended Solid (VSS)	mg/l	$27{,}434 \pm 10.4^a$
Chemical Oxygen Demand (COD)	mg/l	$17{,}480 \pm 17.9^a$
Total Ammonia Nitrogen (TAN)	mg/l NH_3-N	110 ± 0.6^a

[a] Standard Deviation (SD) from the triplicated experiment

2.4 Pretreatment of Aquaculture Sludge by Using Pineapple Enzyme

The pretreatment of aquaculture sludge was conducted to determine the potential of pineapple enzyme on the solubilization of sludge. For the pretreatment of sludge, about 20 ml of the pineapple enzyme solution was diluted with a 200 ml of ultra-pure water to obtain 10% concentration of the enzyme. The pH of the pineapple enzyme solution was adjusted to pH 3.5 and 7.0 with added sodium citrate buffer and phosphate buffer, respectively. The adjusted pH of the enzyme performed in triplicate. Then, to each of the adjusted pH of pineapple enzyme, about 170 ml of aquaculture sludge was added and used for the pretreatment. For control, only 200 ml of the aquaculture sludge was added. The pretreatment of the sludge was incubated in the water bath shaker at agitation 150 rpm by maintaining the temperature 35-37℃ for 5 days (120 hour). The solubility of the sludge was evaluated by determining the TSS and VSS removal efficiencies, and COD and TAN solubilization after treatment.

3.0 RESULTS AND DISCUSSION

3.1 Biocatalytic Activity of Pineapple Enzyme

Biocatalytic activity was determined to evaluate the potential of pineapple enzyme under two pH conditions and as biocatalysis in enzyme reaction. The biocatalytic activity of the pineapple enzyme was determined and the results obtained are illustrated in Figure.1. The pineapple enzyme possessed protease, amylase and lipase activities. As shown in Figure 1, the protease activity at pH 7.0 is higher compared to that at pH 3.5.

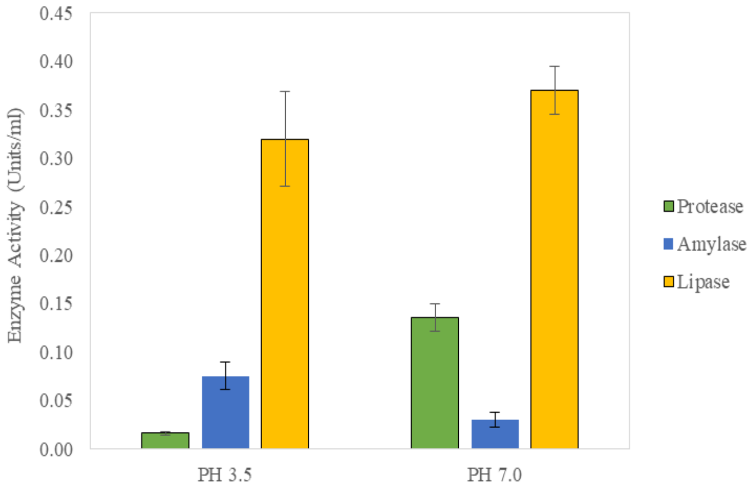

Figure 1: Biocatalytic activity of protease, amylase and lipase with different pH values

According to Arun & Sivashanmugam [9], the biocatalytic activity of protease was able to link tightly to a specific shape of enzyme and its active site of chemical properties, where the alteration of the ionic bond tend to reduce the catalytic function, causing the activity to be higher at pH 7.0. For the amylase activity, different results were obtained for protease and lipase activities, where the pineapple enzyme at pH 3.5 has higher amylase activity, but lower for pH 7.0. This might due to the catalytic property of amylase that is considered to be acidic in nature and an appropriate condition for the enhancement of the enzyme activity. As seen from the results, the lipase activity was slightly higher under both conditions of the pH (7 and 3.5). The results obtained from this study presented for the pineapple enzyme at pH 7.0 showed higher activity compared to at pH 3.5. The pineapple enzyme at pH 7.0 was reported to be the optimal pH for lipolytic activity, similar with the findings from Arun & Sivashanmugam [16]. Lipase activity is usually stable in the pH range of 2.0–9.0 at room temperature [17]. In addition, the structure of the lipase enzyme consists of alkyl groups on the surface, which is known as strongly hydrophobic, causing the lipase activity to be slightly higher for both pH (3.5 and 7.0) [18]. This maximum biocatalytic activity of enzyme was able to degrade high amount of fat and lipid in the sludge.

3.2 TSS and VSS Removal Efficiency

The efficiency of the pretreatment of aquaculture sludge can be predicted based on TSS and VSS removal. TSS and VSS are important to quantify the particulate solid and inorganic solid in the sludge. The effect of pretreatment with pineapple enzyme with pH 3.5 and 7.0 on TSS and VSS removal is shown in Figure 2(a) and Figure 2 (b), with 120 hours pretreatment time. As illustrated in Figure 2(a), TSS removal increment for pineapple enzyme at pH 3.5 and 7.0 was found to be 47% and 55%, respectively. At pH 7.0, it seemed to be higher compared to at pH 3.5. This might due to the presence of organic acid released during the fermentation process that disrupted the extracellular polymeric substance (EPS) in the sludge matrix, causing the cleavage of the cell wall [19]. The sludge structure was destroyed and the organic soluble compound formed into a more soluble compound [20]. Furthermore, the protease activity of the pineapple enzyme at pH higher than 7.0 had triggered the hydrolysis solubility of protein in the sludge. Thus, the TSS and VSS removal efficiencies were found to be higher due to the reduction of solid particulate content in the sludge.

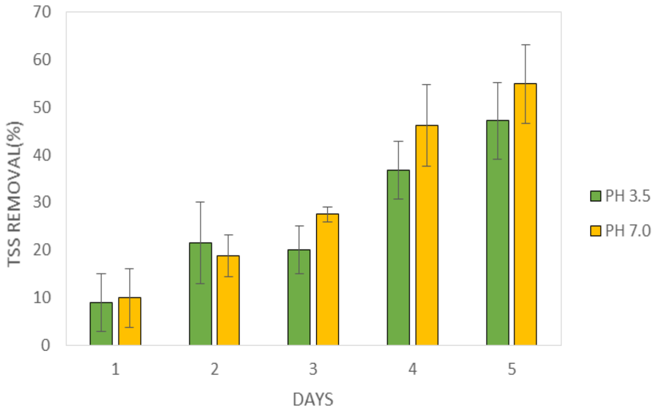

Figure 2 (a): TSS removal efficiency

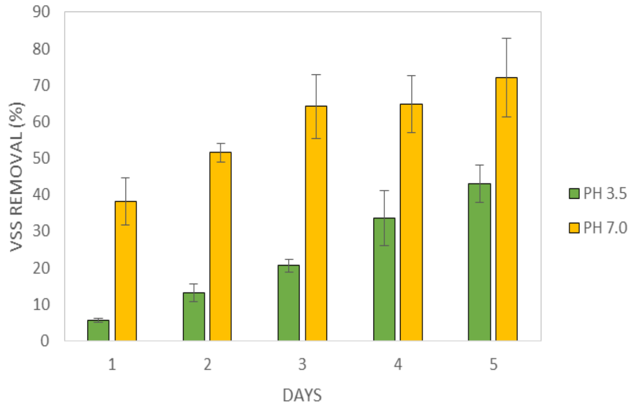

Figure 2 (b): VSS removal efficiency

3.3 Solubilization of Aquaculture Sludge

In order to further improve the biodegradability of the digestion, COD and TAN are important indexes due to the high amount of organic substance found in the aquaculture sludge. The sludge solubilization was analyzed based on COD and TAN solubilization. COD and TAN are determined to evaluate the insoluble organic substance to form into soluble organic substances. Both COD and TAN were analyzed at two different pH of the enzyme, which are pH 3.5 and pH 7.0. From the result observed in Figure 3, the COD solubilizations with treated sludge with pH 3.5 and 7.0 were found to be 84% and 83%, respectively. The hydrolytic of the enzyme degrade the sludge cause increased the COD solubilization and indicated that the organic particle has been solubilized. However, the result obtained for both pH (3.5 and 7.0) of the pineapple garbage enzyme showed no significant difference on the COD solubilization. The findings of this study showed that both pH (3.5 and 7.0) of the enzymes could be applied in the enzymatic pretreatment. The reaction of hydrolytic enzyme of this study was able to hydrolase and break down the insoluble organic substances into soluble organic substances of sludge at both conditions of enzyme's pH. However, according to Sabu, Kiran, & Pandey [21], the activity of the enzyme at pH 3.5 is unstable because the nature of the amino acid at the active site of the enzyme undergoes protonation and deprotonation that ionizes the amino acid and alters the ionic bonds, causing the enzyme to become inactive. However, according to this finding, it might due to higher organic acids as carbon sources found in the pineapple enzyme solution were produced from the ripe pineapple fruits that helped the solubilization of enzyme at pH 3.5 [22].

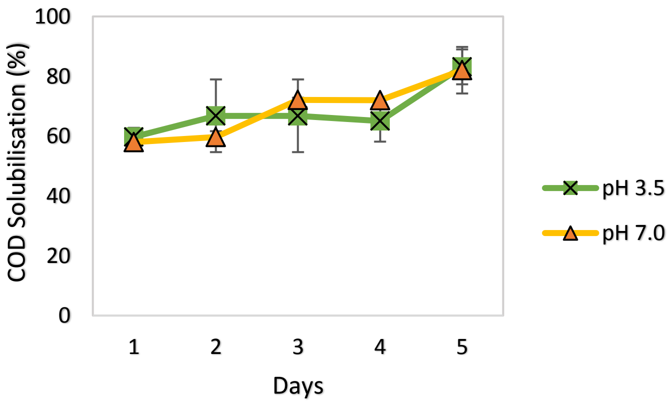

Figure 3: COD solubilization of aquaculture sludge with pineapple enzyme at pH 3.5 and 7.0

As shown in Figure 4, the TAN solubilization increased with treatment time for both pH of garbage enzymes (pH 3.5 and 7). This is because the hydrolytic enzyme reaction occurs to solubilize the organic ammonia, nitrogen and ammonium into a more soluble form [23]. However, present study showed that pH 3.5 has higher TAN solubilization of treated sludge compared that obtained at pH 7.0. Different with previous study Arun & Sivashanmugam [16], pineapple enzyme used at pH 7.0 show highest TAN solubilization of treated waste activated sludge compared with pH 3.5. This might be due to the acidic nature of the enzyme solution added that contained carbon sources produced during fermentation, where it that able to break down the nutrient into a more soluble and simple form [24]. These findings suggest that there could be further improvement of hydrolysis solubility for biogas production via the anaerobic digestion process.

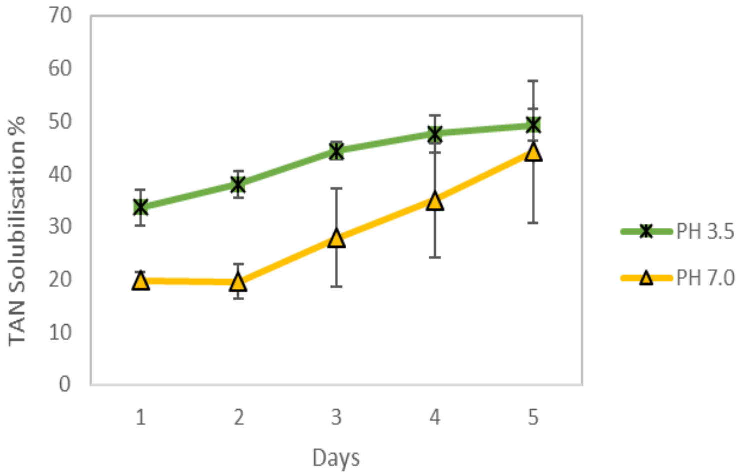

Figure 4: TAN Solubilization on aquaculture sludge with pineapple enzyme at pH 3.5 and 7.0.

4.0 CONCLUSION

In this present study, the enzyme produced from the fermented pineapples waste, namely, as pineapple enzyme was used as enzymatic pretreatment of the aquaculture sludge, which allowed the breakdown of insoluble organic substances of sludge into more soluble substances in the hydrolysis stage of anaerobic digestion process. The results conclude that the biocatalytic reaction of pineapple enzyme was higher for the lipolytic activity at pH 7.0. The TSS and VSS removal efficiencies were up to 55% and 72%, respectively, for pineapples enzyme at pH 7.0. This indicated that the TSS and VSS removal efficiencies of sludge were able to reduce high amount of particulate solid and inorganic solid in the sludge. The solubilization of aquaculture sludge using pineapple enzyme showed that the COD and TAN solubilizations were found to be highest for the pineapple enzyme at pH 3.5 compared to at pH 7.0, and it was found to be highest at 5 days digestion period of the pretreatment. The results obtained from this study clearly showed that the pineapple enzyme has the potential to solubilize the organic compound, which could help to improve the hydrolysis solubility of aquaculture sludge for further investigation of anaerobic biodegradability. Considerably similar study to this work can be applied for other types of fruits and vegetables waste for future research.

Acknowledgements

The authors would like to thank the financial support from the Fundamental Research Grant Scheme (FRGS), Ministry of Higher Education, Malaysia.

References

[1] Anon. 2011. National Agro-Food Policy 2011-2020. Ministry of Agriculture and Agro-Based Industry, Putrajaya, Malaysia. 118.

[2] Anh, P.T., and Mai, H.N.P. 2009. Identify, Evaluate And Prepare Guide Book On Wastewater Treatment Methods For Pangasius Farm. Smart choice Educational and Environmental Consultants prepared for the Sustainable Development of Aquaculture (SUDA). Ministry of Agriculture and Rural Development (MARD), Vietnam.

[3] Im J., Woo, H., Choi, M., Han, and K., Kim C. 2001. Simultaneous Organic And Nitrogen Removal From Municipal Landfill Leachate Using An Anaerobic–Aerobic System. *Water Res*. 35: 2403–2410.

[4] Gerardi, H.M. 2003. The *Microbiology Of Anaerobic Digesters*. John Wiley & Sons, Inc., Hoboken, New Jersey.

[5] Domingues, R.F., Sanches, T., Silva, G.S., Bueno, B.E., Ribeiro, R., Kamimura, E.S., Franzolin, N. R., and Tommaso, G. 2015. Effect of Enzymatic Pre-Treatment On The Anaerobic Digestion Of Milk Fat For Biogas Production. *Food Research International*. 73: 26–30.

[6] Yin, Y., Liu, Y., Meng, S., Kiran, E. U., and Liu, Y. 2016. Enzymatic Pre-Treatment Of Activated Sludge, Food Waste And Their Mixture For Enhanced Bioenergy Recovery And Waste Volume Reduction Via Anaerobic Digestion. *Applied Energy*. 179: 1131–1137.

[7] Masse, L., Masse, D. I., and Kennedy, K.J. 2003. Effect of hydrolysis Pre-Treatment On Fat Degradation During Anaerobic Digestion Of

Slaughterhouse Wastewater. *Process Biochemistry*. 38: 1365-1372.

[8] Wawrzynczyk, J., Szewczyka, E., Norrlowb, O., and Szwajcer, D.E. 2007. Application of Enzymes, Sodium Tripolyphosphate And Cation Exchange Resin For The Release Of Extracellular Polymeric Substances From Sewage Sludge: Characterization Of The Extracted Polysaccharides/Glycol Conjugates By A Panel Of Lectins. *Journal of Biotechnology*. 130: 274–281.

[9] Arun, C. and Sivashanmugam, P. 2015. Investigation of Bio Catalytic Potential of Garbage Enzyme and Its Influence on Stabilisation of Industrial Waste Activated Sludge. *Process Safety Environment*. 94: 471-478.

[10] Nazim, F., and Meera, V. 2013. Treatment of Synthetic Greywater Using 5% And 10% Garbage Enzyme Solution. *Bonfring International Journal of Industrial Engineering and Management Science*. 3: 111-117.

[11] Tang, F.E., and Tong, C.W. 2011. A Study Of The Garbage Enzyme's Effects In Domestic Wastewater. *International Journal of Environment, Chemical, Ecological, Geological and Geophysical Engineering*. 5: 887-892.

[12] American Public Health Association (APHA), 2005. Standard Method for Examination of Water and Waste Water. 21st ed.APHA, Washington, DC.

[13] Pandey, A, Benjamin, S., Soccol C. R., Nigam, P. and Kriege,r N., 1999. The Realm of Microbial Lipases in Biotechnology. *Biotechnology Applied Biochemistry*. 29: 119-131.

[14] Benfeld, P. 1951. Enzymes of Starch Degradation and Synthesis. *Advance in Enzymology*. 12: 379-428.

[15] Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein Measurement with the Follin Phenol Reagent. *Journal of Biology. Chemistry*. 193: 265–275.

[16] Arun, C. and Sivashanmugam, P., 2015. Solubilisation of Waste Activated Sludge Using a Garbage Enzyme Produced From Different Pre-consumer Organic Waste. *Journal of Royal Society of Chemistry*. 5: 51421-51427

[17] Ribeiro, B. D., Castro, A. M. De, Alice, M., Coelho, Z., and Guimar, D. M. 2011. Production and Use of Lipases in Bioenergy : A Review from the Feedstocks to Biodiesel Production. *Enzyme Research*. 2011:1-16.

[18] Lívia, T.A.S., Jamil, S.O., Vera, L.S., Wiliam, C.B.R., Marcelo, M.S., and Rodrigo, R.R. 2014. Lipolytic Potential of Aspergillus japonicus LAB01 : Production, Partial Purification, and Characterisation of an Extracellular Lipase. *BioMed Research International*. 2014: 1-11.

[19] Guo, J.S., and Xu, Y.F. 2011. Review of Enzymatic Sludge Hydrolysis. *Journal of Bioremediation Biodegradation*. 2(5): 1-7.

[20] Uma Rani, R., Adish Kumar, S., Kaliappan, S., Yeom, I.T., and Rajesh Banu, J. 2014. Enhancing the Anaerobic Digestion Potential Of Dairy Waste Activated Sludge By Two Step Sono-Alkalization Pre-Treatment. *Ultrasonic Sonochemistry*. 21: 1065-1074.

[21] Sabu, A., Kiran, G.S., and Pandey, A. 2005. Purification and Characterization of Tannin Acyl Hydrolase from Aspergillus Niger ATCC 16620. *Food Technology and Biotechnology*. 43: 133-138

[22] Ketnawa, S., Chaiwu, P. and Rawdkuen, S. 2012. Pineapple Wastes: A Potential Source for Bromelain Extraction. *Food and Bioproducts Processing*. 90: 385–391.

[23] Li, X., Peng, Y., He, Y., Jia, F., Wang, S., and Guo, S. 2016. Applying Low Frequency Ultrasound On Different Biological Nitrogen Activated Sludge Types: An Analysis Of Particle Size Reduction, Soluble Chemical Oxygen Demand (SCOD) And Ammonia Release. *International Bio deterioration & Biodegradation*. 112: 42-50.

[24] Oyedeji, O., Bakarea, M.K., Adewaleb, I.O., Olutiolaa, P.O., and Omoboyea O.O. 2017. Optimized Production and Characterization of Thermostable Invertase from Aspergillus Niger IBK1, Using Pine