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PRODUCTION AND CHARACTERIZATION OF BIO CATALYTIC ENZYME PRODUCED FROM FERMENTATION OF FRUIT AND VEGETABLE WASTES AND ITS INFLUENCE ON AQUACULTURE SLUDGE

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Abstract

The aquaculture sludge discharged from shrimp aquaculture industry may result in high organic content and nutrients that are considered as a source of surface and ground pollution. Hence, before disposing, it has to be treated efficiently to reduce its pollutants concentration. Anaerobic digestion technology has been commonly applied for sludge treatment but it requires longer retention time and the efficiency for the overall organic matter degradation is relatively low. Therefore, a pretreatment is usually applied before further anaerobic treatment. This study focused on the production of bio catalytic enzyme from fermentation of fruit and vegetable wastes such as pineapple, mango, orange and tomato dregs to increase the aquaculture sludge stability. The solution of the wastes is known as garbage enzyme. It was produced by three *months' fermentation of molasses, waste and water mixture with the ratio of 1:3:10. Characterization of the enzyme showed that it possessed lipase, amylase and protease activities. All types of fruit and vegetable wastes were pretreated individually at pH 7.0 within the duration of 120 hours and were labelled as pineapple garbage enzyme (PGE), mango garbage enzyme (MGE), orange garbage enzyme (OGE) and tomato garbage enzyme (TGE). The efficiency of the pretreatment was evaluated based on the removal of total suspended solid (TSS), volatile suspended solid (VSS), chemical oxygen demand (COD) and total ammonia nitrogen (TAN). A higher removal of all parameters was observed in all pretreated experiments. The pineapple garbage enzyme performed a greater reduction of TSS and VSS within the range of 54 percent to 72 percent. COD removal efficiency was higher in pineapple and orang garbage enzyme with 82 percent and 88 percent removal, respectively. While for TAN, orange garbage enzyme showed a higher reduction with 65 percent removal. These findings proved that enzyme solution produced from fruit and vegetable wastes have the potential to pretreat aquaculture sludge when the reduction of selected parameters were observed and this will help reducing shock lock or inhibition prior to anaerobic digestion.*

Keywords

Enzyme, Fermentation, Fruit and Vegetable Wastes, Pretreatment, Aquaculture Sludge

1. Introduction

Aquaculture industry in Malaysia is becoming a major contribution to the nation's economy. According to Yusoff (2015), the population increase in Malaysia subsequently increases the demand for fish as main protein source. The National Agro-food Policy (2011-2020) has estimated that the yearly demand of fish from year 2011 to 2020 will increase from 1.7 to 1.93 million tons. To sustain the growth, the aquaculture industry has been exploited and expanded for downstream activities such as feed mills, fish seed production, fish processing plants, and other relevant industries. Aquaculture sludge is the waste resulted from fish farming activities that caused deposits at the bottom of the water bodies. It is approximately 25 percent of unconsumed animal foods and 27 percent of the foods become their excrement and these turn into sludge (Timmons & Ebeling, 2007; Mirzoyan, Tal, & Gross, 2012). The high pollutant content discharge from the activities is known as aquaculture sludge which resulted in high organic content and nutrients that are considered as a source of surface and ground pollution.

Thus, before disposing the sludge to the watercourses, it has to be treated efficiently to reduce its pollutant concentration.

Anaerobic digestion is one of the technology approaches to treat aquaculture sludge into valuable by-product such as biogas. The biogas production via anaerobic digestion offers most environmental-friendly option for waste recovery concept. The four reactions that occurred during the entire process of anaerobic digestion to form methane are hydrolysis, acidogenesis, acetogenesis and methanogenesis stages. All these four reaction occurred simultaneously and independent of each other. But, there is a limitation step during hydrolysis stage where intermediate of the conversion of non-soluble biopolymers to soluble organic compounds may produce (Chen, Cheng, & Creamer, 2008). To overcome the limitation, various pretreatment technologies and methods have been invented in recent years to improve the effectiveness of further anaerobic digestion process. Solubilization is a biological process that solubilizes organic matter to more soluble form, thus it can be treated directly by the anaerobic microbes subsequently (Arun & Sivasmugam, 2015). Through pretreatment process, dissolved constituents will induce hydrolysis acceleration rate hence, improvement in biodegradability, sludge dewatering and pathogen reduction also can be achieved (Muller, 2000).

Pretreatment methods including physical (mechanical, thermal, ultrasonic, microwave), chemical (alkaline, ozone oxidation) and biological (enzyme) have been demonstrated to yield positive effects on anaerobic digestion (Yi, Han, & Zhuo, 2013). As physical and chemical pretreatment require higher energy, chemical addition and high costs to operate and handle, enzyme pretreatment is becoming a preferable method due to its eco-friendly and low operating cost features. Enzymatic pretreatment was demonstrated to be an effective treatment to increase the solubility of the selected inhibitor in several types of wastewater. Hydrolytic enzymes such as amylase, lipase and protease are amongst many commercial enzymes used in improving sludge solubility. For instance, the characteristics of sewage sludge improved in terms of solid and pathogen reduction when pretreated with alkaline protease (Parmar, Singh, & Owen, 2001). The enzymatic pretreatment of lipase had been employed to overcome sludge problems from dairy wastewater treatment and had significantly reduced its fat content (Adulkar & Rathod, 2013). A commercial enzyme application on pretreatment of sludge requires high costs and for large scale industry, it will be less economical. Thus, there is a need to produce a low-cost enzyme production which can be produced from fruit and vegetable wastes.

There's a product developed in 2006 by Dr. Rosukon, a researcher from Thailand, used organic solid waste and named the obtained solution as garbage enzyme (Arun & Sivashanmugam, 2015). Garbage enzyme is produced through the fermentation of the mixture of brown sugar, water, kitchen waste or fresh vegetable or fruit waste (Nazim & Meera, 2013), and according to Tang & Tong (2013), the process requires three months. The applications of garbage enzyme on several sludge and wastewater characteristics have been demonstrated in recent years. The garbage enzyme plays an important role to achieve a degree of degradation similar to the performance of commercial enzyme. Nazim and Meera (2013) produced garbage enzyme and used it to treat synthetic grey water with 5 percent and 10 percent dilution to wastewater and the treatment successfully removed phosphorus and nitrogen. Meanwhile, Tang and Tong (2011) studied the influence of garbage enzyme on domestic wastewater and it was observed that total dissolved solids (TDS) and COD were removed within the range of 20 to 60 percent when pretreated with 9 percent of garbage enzyme. Arun and Sivashmugam (2015) investigated the solubility of waste-activated sludge using garbage enzyme were increased and the pretreatment had showed a reduction of such parameters (25 percent COD, 20 percent total nitrogen (TN) and 11 percent total phosphorus (TP)). Furthermore, the garbage enzyme acts not only as additives to treat specific pollutant, but also reduces the disposal of solid waste generated.

Therefore, in the present work, to accomplish the concept of low-cost waste recovery strategy, resources used in this study were taken from the commercial area that are no longer used. Garbage enzyme was produced by utilizing fruit and vegetable wastes such as pineapple, mango, orange and tomato dregs, which undergo fermentation process. After fermentation, the characteristics of garbage enzyme were evaluated including its bio-catalytic activities. Then, pretreated experiments were performed to determine the influence of individual garbage enzyme produced on the improvement of aquaculture sludge organic concentration. Parameter before and after treatment for TSS, VSS, COD and TAN were evaluated.

2. Materials and Method

2.1 Garbage Enzyme Production and Characterization

The fruit and vegetable wastes such as pineapple, mango, orange and tomato dregs were collected from supermarket waste near Kuala Terengganu, located at the east coast of Peninsular Malaysia. The wastes were fermented separately in 5L airtight container and the individual garbage enzyme were labelled as pineapple enzyme (PGE), mango enzyme (MGE), orange enzyme (OGE) and tomato enzyme (TGE). In each container, 1 part of molasses, 3 parts of each type of wastes (PGE, MGE, OGE & TGE) and 10 parts of water were added, respectively. The containers were placed in a cool and dry area and were fermented for a durations of three months. The gas formations were released daily for the first month and once a month for the next two months. After three months fermentation, all solutions were filtered and centrifuged at 3000 rpm for 30 minutes and stored at 4 ºC prior to use.

The characterizations of each enzyme were conducted based on parameters such as bio catalytic activities, pH, total solid (TS), volatile solid (VS), total suspended solid (TSS), volatile suspended solid (VSS), chemical oxygen demand (COD), total ammonia nitrogen (TAN) and citric acid. Except for bio catalytic activities and citric acid, all procedures for each analysis were carried out based on the standard method (APHA, 2005). Bio catalytic characterizations were determined for each enzyme to assure the solution produced from fermentation process contained cell-free enzymes activities such as lipase, amylase and protease. The citric acid concentration was determined by using High Performance Liquid Chromatography (HPLC).

2.2 Lipase Activity

Lipase activity was determined by using spectrophotometer and method suggested by Pandey, Benjamin, Soccol, Nigam, & Krieger (1999). A mixture containing 0.05 ml of individual eco-enzyme and 0.95 ml of substrate (1:9 of 3.0Mm p-NPPin 2 propanol and 0.4 percent Triton X100 and 0.1 percent Arabic gum) was prepared. Then, the mixture was incubated for 20 minutes at 37 ºC and the reading was evaluated by using absorbance at 440 nm. The lipase activity was expressed by the amount of 1 mole of *p*-nitrophenol released per minute of tyrosine.

2.3 Amylase Activity

A method established by Benfeld (1951) was used to measure amylase activity. The mixture of 0.50 ml of individual eco-enzyme and 0.50 ml of 1 percent starch solution was incubated for 10 minutes at 25 ºC. Then 1 ml of dinitrosalicylic acid color reagent was added. The mixture was then incubated at boiling water bath for 5 minutes to allow reaction and cooled to room temperature. Finally, the absorbance was read at 540 nm. The amylase activity was measured by the reduction of 3,5-dinitrosalicylic acid.

2.4 Protease Activity

Protease activity was experimented by using the method introduced by Lowry, Rosebrough, Farr, & Randall (1951). 1 ml of individual eco-enzyme was mixed with 1 ml of 2 percent casein solution and pre-warmed for 10 minutes to allow for the reaction to occur. Then, the reaction was stopped by adding 2 ml of trichloroacetic acid solution and incubated for 10 minutes at 35 ºC. After incubation, the solution was centrifuged at 3000 rpm and 1 ml supernatant was taken. 5 ml of Na_2CO_3 and 1 ml of Follin phenol reagent were added to the supernatant. Finally, the absorbance was read at 660 nm. The activity of protease was signified as amount of enzyme that released 1 mg of tyrosine equivalent per minute.

2.5 Aquaculture Wastewater Characterization

The aquaculture sludge was collected from shrimp hatchery located in Setiu, Terengganu, Malaysia and was kept at 4 ºC prior to use. The characteristic of aquaculture sludge were then analyzed according to pH, TS, VS, TSS, VSS, COD and TAN as shown in Table 1.

Table 1: *Characteristic of Aquaculture Wastewater*

Parameter	Units	Concentration
pH	-	6.50 ± 0.2
TS	%	5 ± 0.1
VS	%	39 ± 0.2
TSS	mg/l	57,450 ± 17.0
TDS	mg/l	705,717 ±10.4
VSS	mg/l	27,434 ± 10.4
COD	mg/l	17,480 ± 17.9
TAN	mg/l NH_3-N	110 ± 0.6

2.6 Pre-treatment of Aquaculture Wastewater using Different Types of Garbage Enzyme

A series of pre-treatment experiments were conducted to study the influence of individual eco-enzyme on the pretreatment of aquaculture sludge. For the pre-treatment, 20 ml of each PE, ME, OE and TE were diluted with 200 ml of ultra-pure water. pH of each garbage enzyme was adjusted to 7.0 by adding phosphate buffer solution. According to the previous study by Arun & Shivanmugan (2015) and Panda, Mishra, Kayitesi, & Ray (2016), pH is one of the important factors affecting the hydrolytic enzyme activities in order to reduce and suppress enzyme mechanisms in acidic condition and it showed higher reduction on the pH level. Garbage enzyme with adjusted pH was used for the pretreatment for COD and TAN improvement. Then, four conical flasks were added with 150 ml of aquaculture sludge each and followed by the addition of 50 ml individual garbage enzyme and labelled accordingly. A control experiment was also conducted for comparison. In control flasks, only aquaculture sludge was added. After that, all experiments were prepared in triplicate and were agitated at 100 rpm with temperature of 35 to 37 ºC for 5 days (120 hours) in an incubator shaker. The efficiency of the pretreatment was evaluated based on TSS, VSS, COD and TAN removal.

3. Results and Discussion

3.1 Characteristics and Bio Catalytic Activity in Different Types of Garbage Enzyme Solutions

Individual garbage enzyme solution characteristics after three months of fermentation period are shown in Table 2. pH of all enzymes are acidic in nature within the range of 2.8 - 3.3 due to the presence of organic and volatile acid in the solution. During the fermentation process, the volatile acids were converted from carbohydrates and the organic acids were extracted from the fruit dregs into the enzyme solution (Arun & Sivashanmugam, 2015). Report by Selvakumar & Sivashanmugam (2017) mentioned that the vegetable dregs and fruit peels are considered as organic solid waste. During the fermentation process, brown sugar was added as a main substrate and it was expected to contain high organic matter (Tang & Tong, 2011). According to Ademollo et al. (2012), organic substances in the water body are subjected to a partition between suspended and dissolved solid phases. As a result, garbage enzyme possesses greater amount of COD, TS, TDS and TSS.

Table 2: *Characteristics of Garbage Enzyme Solution*

Parameters	PE	ME	OE	TE
pH	3.30 ± 0.03	2.95± 0.06	3.25± 0.06	2.80 ± 0.01
TSS (mg/L)	897.67 ± 57.74	567.67± 11.55	600.67± 20	455.67± 17.32
VSS (mg/L)	512.67± 28.87	497.67± 20.82	488.67± 10	412.67± 5.77
COD (mg/L)	7160± 100	5660± 200	7020± 100	7110± 100
Citric Acid (mg/L)	45.01±1.45	19.92±0.03	29.18±0.86	16.41±1.06

The results for bio catalytic activity of individual garbage enzyme (PGE, MGE, OGE & TGE) are shown in Figure 2. The figure depicted that all types of garbage enzyme at both pH 3.5 and 7.0 possess protease, amylase and lipase activity. However, it can be seen that higher concentrations of bio catalytic enzyme activities were recorded in pH 7.0 as compared to pH 3.5 for all types of garbage enzyme. It is shown that PGE has higher protease activity at pH 7.0 but lower at pH 3.5. At pH 7.0, protease activity is the highest for pineapple garbage enzyme and the lowest for mango garbage enzyme. According to Arun & Sivashanmugam (2015), the optimal pH for protease to function must be in the range between 6 and 7. The ability of protease to catalyze a substrate is highly related to its specific shape and chemical properties of active site at optimal pH.

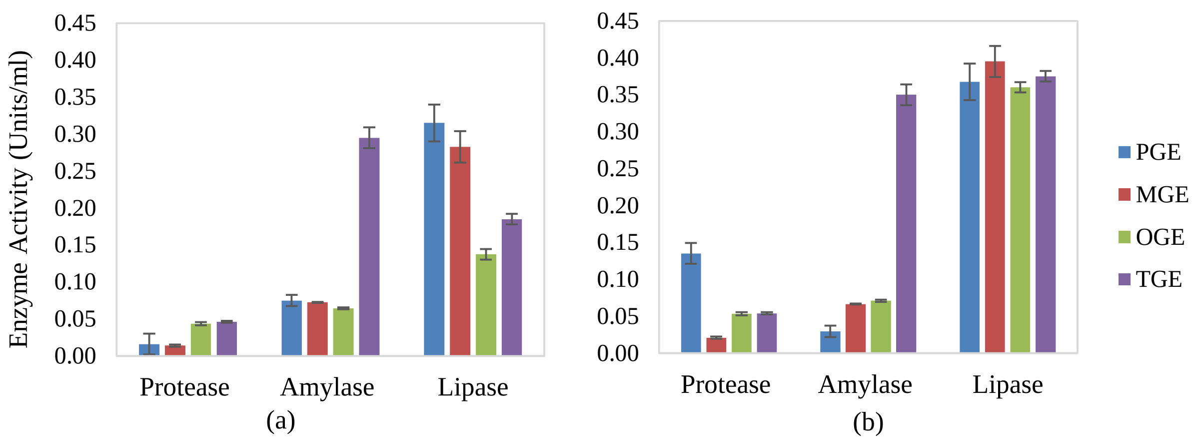

Figure 1: *Individual bio catalytic enzyme (PGE, MGE, OGE & TGE) at (a) pH 3.5 and; (b) pH 7.0*

For amylase activity, tomato garbage enzyme showed highest concentration in pH 7.0 while the lowest concentration was observed from pineapple garbage enzyme. According to Arun & Sivashanmugam (2015), same as protease, the catalytic property of amylase is commonly higher at pH within the range of 6 to 7. Therefore, the observed result indicated that maintaining pH of garbage enzyme within the range will achieve higher amylase activity of the enzyme. Lipase activity were comparable and higher in all garbage enzyme as compared to protease and amylase. According to Shu, Xu, & Lim (2006), lipase activity is generally high within the range of 7 to 10. In order to achieve higher lipolytic activity, pH of garbage enzyme should be maintained within the range of 7 and 8 as stated by Arun & Sivashanmugam (2015). This finding is in accordance as what has been observed by Shu et al. (2006) and Arun & Sivashanmugam (2015). Thus, these findings confirm the presence of bio catalytic enzyme activities in all types of garbage enzyme experimented in this study and pH 7.0 was used for further pretreatment in order to determine its influence on aquaculture sludge.

3.2 Reduction of TSS and VSS

The reductions of TSS and VSS were evaluated to determine the efficiency of the treatment to stabilize the aquaculture sludge. These reductions on aquaculture sludge after being pretreated with different types of garbage enzyme are depicted in Figure 3.2.

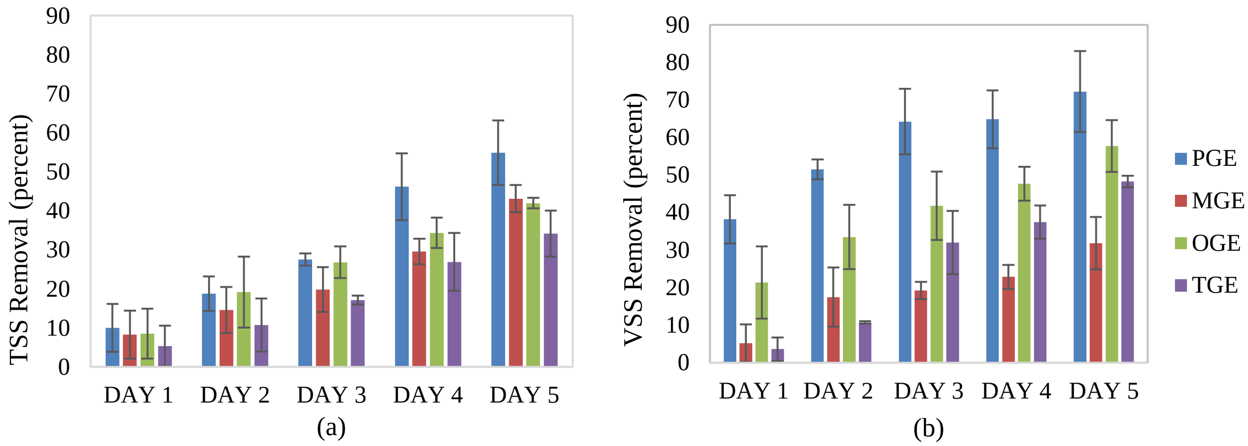

Figure 2: *Effects of a) TSS and; b) VSS removal on aquaculture sludge pretreatment with garbage enzyme (PGE, MGE, OGE & TGE)*

It was observed from those figures that the reductions of TSS and VSS increased when the treatment time increased until day 5 (120 hours). Results showed that the TSS removal of PGE, MGE, OGE and TGE pretreatment were 54 percent, 43 percent, 41 percent and 34 percent, respectively while for VSS, the removal of PGE, MGE, OGE and TGE pretreatment were 72 percent, 32 percent, 58 percent and 48 percent, respectively. High removal percentage of TSS and VSS in enzyme pretreatment with pH 7.0 was due to the neutral condition of the process might have induced the treatment mechanism by garbage enzyme. According to Yang et al. (2010), acidic condition of the pretreatment will suppress secreted immobilized bacteria in the enzyme. Thus, the mechanism of destroying sludge solid structure by organic component into the soluble part will be dissuaded. Previous study evaluated by Yang et al. (2010), demonstrated that the excess sludge treatment with protease and amylase had shown VSS reduction within the range of 40 to 54 percent. In this study, the reduction of TSS and VSS were highest in PGE pretreatment. This might be due to the organic acid content in PGE is higher than in other wastes. Citric acid was the main organic acid in PGE produced from fermentation process as what has been observed in previous study by Arun & Sivashanmugam (2015). According to Foladori, Andreottola, & Ziglio (2010), this hydrolytic enzyme that contains higher citric acid level than other enzymes has the potential to break more sludge matrix and this will result in higher TSS and VSS removal.

3.3 Reduction of COD

The effects of all garbage enzyme produced (PGE, MGE, OGE and TGE) on COD removal are demonstrated in Figure 3.3. The COD is becoming an important index to evaluate the aquaculture sludge pretreatment efficiency with regards to improve further biodegradability and bioavailability. The increase of COD removal could efficiently improve the aquaculture sludge biodegradation for further anaerobic treatment and hence reduce the impact of shock load into the system (Yang et al., 2010). In this study, COD removal in all types of garbage enzyme pretreatment increased throughout the treatment time. The results showed that the COD removal of OGE, PGE, TGE and MGE were found to be at 88 percent, 82 percent, 78 percent and 63 percent respectively.

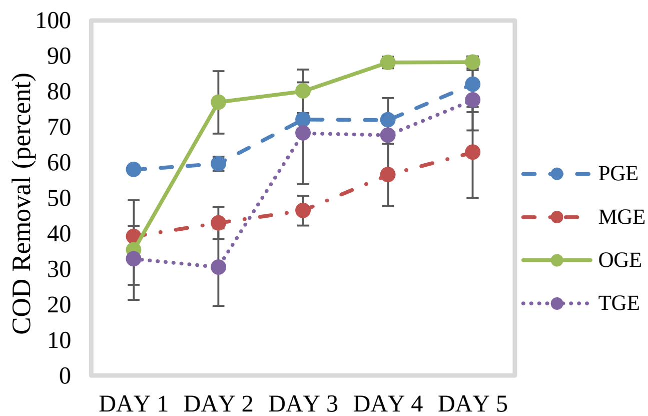

Figure 3: *COD removal on aquaculture sludge pretreatment with garbage enzyme (PGE, MGE, OGE & TGE)*

COD removal of OGE at pH 7.0 showed the highest percentage when compared to other types of garbage enzyme. This is due to the presence of citric acid in orange peels. The acidic nature of the orange peel which might has contributed for the breakdown from insoluble to soluble form (Arun and Sivashmugam, 2015).

3.4 Reduction of TAN

In aquaculture system, large amount of nutrient concentrations such as feed residue and fish excreta will be accumulated in the form of organic nitrogen, ammonia and ammonium, which were insoluble substances (Kuusik, 2014). To evaluate the insoluble substances to form into soluble substances, TAN removal was determined. As seen in Figure 3.4, the increase of TAN removal was obtained from all garbage enzyme pretreatment. Highest TAN removal which is 65 percent was observed from OGE pretreatment. This finding is inclined with previous study from Arun and Sivashanmugam (2015), whereas OGE showed increased values in TAN solubilization when garbage enzyme was treated with waste activated sludge (WAS). The increase in TAN removal is due to the presence of carbon sources presence in the garbage enzyme. The carbon-based particulate will be converted from insoluble substance to soluble substance with the increase in pretreatment time (Ushani, Rajesh Banu, Kavitha, Kaliappan, & Yeom, 2017).

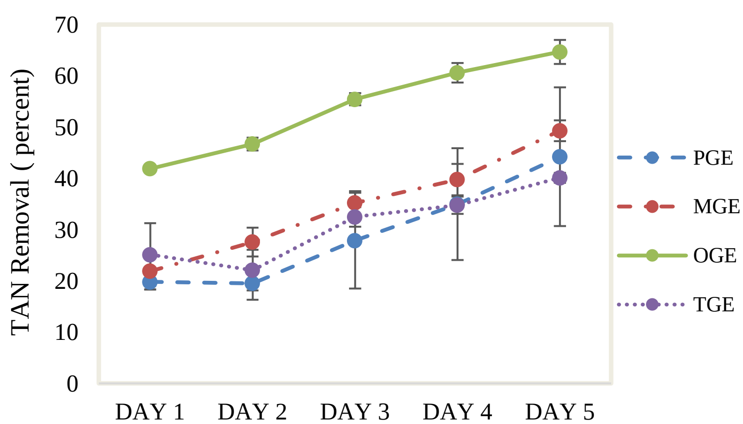

Figure 4: *TAN removal on aquaculture sludge pretreatment with garbage enzyme (PGE, MGE, OGE & TGE)*

4. Conclusions

The utilization of the garbage enzyme produced from fruit and vegetable wastes such as pineapples, mangoes, oranges and tomatoes were used to determine the bio catalytic activity and its influence on solubilization of aquaculture sludge. Thus, these findings confirmed that PGE, MGE, OGE and TGE possess lipase, amylase and protease activities which are in accordance to the findings from Arun & Sivashanmugam (2015). The TSS and VSS reductions showed higher reduction for PGE pretreatment while COD and TAN removal were found to be the highest for OGE. The increase in removal efficiency of the aquaculture sludge obtained from this study showed that the fruit and vegetable wastes have the potential in the breakdown of the selected pollutants, thus promoting the improvement of aquaculture sludge quality that will subsequently enhance degradation process during hydrolysis stage, and thus might improve the rate limiting stage for further anaerobic digestion. Previous studies by applying garbage enzyme for the treatment of different kinds of waste water including waste activated sludge, synthetic greywater and domestic wastewater had also strongly recommended the ability of garbage enzyme in the removal of selected pollutants (Tang & Tong, 2011; Nazim & Meera, 2013; Arun & Sivashanmugam, 2015).

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