Investigation on the Influence of Bio-catalytic Enzyme Produced from Fruit and Vegetable Waste on Palm Oil Mill Effluent

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Abstract. Pre-consumer waste from supermarkets, such as vegetables and fruits dreg are always discarded as solid waste and disposed to landfill. Implementing waste recovery method as a form of waste management strategy will reduce the amount of waste disposed. One of the ways to achieve this goal is through fermentation of the pre-consumer supermarket waste to produce a solution known as garbage enzyme. This study has been conducted to produce and characterize biocatalytic garbage enzyme and to evaluate its influence on palm oil mill effluent as a pre-treatment process before further biological process takes place. Garbage enzyme was produced by three-month long fermentation of a mixture of molasses, pre-consumer supermarket residues, and water in the ratio of 1:3:10. Subsequently, the characterization of enzyme was conducted based on pH, total solids (TS), total suspended solids (TSS), total dissolved solids (TDS), chemical oxygen demand (COD), and enzyme activities. The influence of produced enzyme was evaluated on oil & grease (O&G), TSS and COD of palm oil mill effluent (POME). Different levels of dilution of garbage enzyme to POME samples (5%, 10%, 15%) were explored as pre-treatment (duration of six days) and the results showed that the garbage enzyme contained biocatalytic enzyme such as amylase, protease, and lipase. The pre-treatment showed removal of 90% of O&G in 15% dilution of garbage enzyme. Meanwhile, reduction of TSS and COD in dilution of 10% garbage enzyme were measured at 50% and 25% respectively. The findings of this study are important to analyse the effectiveness of pre-treatment for further improvement of anaerobic treatment process of POME, especially during hydrolysis stage.

1. Introduction
Fruit markets, vegetable markets, restaurants, and food processing industries are the prime contributors to pre-consumer type waste such as vegetables, fruits, and its peels in large quantities [1]. Sixty percent of pre-consumer type waste produced by food processing industries is made up of organic matters [2]. At present, the management of such organic waste is a significant global issue. Value added bio-products can be produced from the organic waste which also reduce the emission of greenhouse gas when disposed to landfill [1].

In 2006, a researcher from Thailand named Rosukun developed a solution form product using organic solid waste and named it garbage enzyme [1]. This enzyme is a composite organic substance made up of organic acids, protein chains (enzyme), and mineral salts produced by the fermentation of waste vegetables, fruits or its peels, sugar, and water. The garbage enzyme can be applied to compose, decompose, transform, and catalyze [3]. Besides, this enzyme solution works in the same way as...
commercial hydrolytic enzyme as it is able to achieve a high level of degradation within a short period. Thus, it can be utilized as a low-cost alternative to improve wastewater treatment processes through the removal of impurities, harmful sludge, and bacteria, which in turn promotes recycling of waste back into the earth [4]. Garbage enzyme is produced by the fermentation of kitchen waste or fresh vegetable waste, such as fruit and vegetable peels, water, and brown sugar [5]. The process for fermentation takes three months [4]. There are protease, amylase, and lipase activity in garbage enzyme which can be used to degrade proteins, carbohydrates, and lipids respectively. Garbage enzyme also possesses pathogen-killing or pathogen-inhibiting properties [1].

This study is focused on the implementation of low-cost pre-treatment using garbage enzyme and its effectiveness on palm oil mill effluent (POME). Palm oil is one of the world’s most rapidly expanding equatorial crops [6]. POME is the organic mud-like waste produced from the processing of fresh fruit bunches (FFB) into crude palm oil (CPO) [7]. If discharged to the environment directly without any treatment, the wastewater will cause significant pollution. The common method used to treat POME in Malaysia is anaerobic digestion [8, 9].

Long chain fatty acid (LCFA) is the intermediate compound generated from the hydrolysis of O&G during anaerobic digestion of wastewater [10, 11]. LCFA produced is able to inhibit the activity of various microorganisms. The inhibition can result in the failure of the LCFA fermentation and cause a noticeable lag phase in anaerobic process [10, 12, 13]. High amounts of total solids will reduce substrate degradation in anaerobic digestion and will consequently decrease biogas production [14].

Thus, the purpose of pre-treatment is to reduce inhibition effect resulting from POME degradation and to further enhance the treatment process during anaerobic digestion. The garbage enzymes have similar functions as commercial enzymes in accomplishing a higher degree of degradation within a shorter period of time [4, 15]. The aim of this study was to analyze the effects of enzymatic pre-treatment on POME using garbage enzyme by contributing to the improvement of further anaerobic treatment process, especially during hydrolysis stage.

2. Materials and Method

2.1 Sampling
POME samples were collected from the discharge of a palm oil mill plant located in Kemaman, Terengganu.

2.2 Garbage Enzyme Preparation
The fruit and vegetable dregs were collected from pre-consumer supermarket residues consisting of orange, pineapple, tomato, and mango dregs. The ingredients needed to prepare the garbage enzyme were molasses, fruit and vegetable dregs, and water. They were mixed together in a plastic container in the ratio of 1: 3: 10. During the first month, gases produced were released daily. The containers were kept in a cool and dry area. After three months of fermentation, the enzyme produced was filtered to remove the residue.

2.3 Characterization of Garbage Enzyme
The characterization of garbage enzyme was investigated based on parameters such as pH, total solids (TS), total dissolved solids (TDS), total suspended solids (TSS), and chemical oxygen demand (COD). Procedures in standard method (APHA, 2005) and enzyme activities (protease, amylase, and lipase) method were determined by using Casein Digestion Unit Analytical Method (CDU), 3,5-dinitrosalicylic Acid (DNS) Method, and Titrimetric Method.

2.4 Batch Test
Batch tests were conducted to determine the effective dosage of garbage enzyme for the treatment of POME. For this study, 5%, 10% and 15% levels of dilution of garbage enzyme to POME sample were
selected. The mixture of garbage enzyme with POME sample was allowed to digest for a period of six days, to allow the enzyme to affect the POME sample. Water quality parameters (TSS, COD, O&G) were monitored and tested daily to study the effects of degradation of the wastewater sample.

3. Results and Discussion

3.1 Characteristics of Garbage Enzyme

During the process of fermentation, carbohydrates were converted into volatile acids. Organic acids present in fruit dregs also leached out into the solution. Therefore, garbage enzyme is acidic in nature due to the presence of volatile acid and organic acid in the solution [1]. COD is a method used to evaluate the total amount of organic matter. It is the main parameter used to determine the organic content in the wastewater sample [16, 17]. As garbage enzyme is produced from kitchen waste and brown sugar is added as fermentation substrate, it is expected to contain high amounts of organic matter [4]. Therefore, as shown in Table 3.1, COD of garbage enzyme produced is high. Vegetable dregs and fruit peels are considered as organic solid waste [3]. Organic substances in the water body are subjected to a partition between suspended and dissolved solid phases [18]. Therefore, as tabulated in table 1, the amount of TS, TDS, and TSS is high in garbage enzyme.

Table 1. Characteristics of Garbage Enzyme

| Parameter | Value         |
|-----------|---------------|
| pH        | 3.65          |
| TS        | 33,789.33     |
| TDS       | 27,066.67     |
| TSS       | 2,893.33      |
| COD       | 121,636.70    |

*Units in mg/L except pH

3.1.1 Enzyme Activities in Garbage Enzyme

Figure 1 reveals that the lipase activity reached maximum at pH 7. The pH of garbage enzyme should be maintained between the range of pH 7 and 8 in order to achieve higher lipolytic activity [1]. Figure 2 shows that protease activity was higher at pH 7. The pH of garbage enzyme should be maintained within pH 6.5 and 7 to achieve higher proteolytic activity [1]. It is observed from figure 3 that the amylase activity is higher at pH 7.5. The catalytic property of amylase is commonly higher at the range of pH 6 to 7 [1]. These results indicate that the process of fermentation of fruit and vegetable dregs have the potential to produce a solution that contains biocatalytic enzyme known as garbage enzyme.

Figure 1. Lipase Activity in Garbage Enzyme at Different pH Levels
3.2 Characteristics of POME
Table 2 sets out the characteristics of POME as measured in this study. It is observed that the concentration of COD in POME is significantly high. In most cases, the total amount of organic pollutants contained in wastewater can be determined by the application of COD [19]. Due to the presence of unrecovered palm oil in POME, the raw or partially treated POME contains extremely high amounts of degradable organic matter [20]. In this study, the BOD value of POME is considered high. BOD is also commonly used to assess the amount of organic pollution in wastewater sample by measuring the oxygen demand of both organism and organic matter in the POME. It is basically used to determine the approximate amount of oxygen required by the microorganisms and bacteria while inducing the degradable organic matter contained in the sample [21]. Untreated POME contains residual oil which requires a high amount of oxygen before it can be decomposed entirely [22]. POME contains considerable quantities of solids [23]. These suspended solids mainly originate from palm mesocarp, also known as cellulose fruit debris [24]. There are inert suspended solids that will result in bottom sediments [25]. There are high amounts of oil and grease in POME. The O&G of POME can be found in two phases [26]. They are suspended in the supernatant as emulsions and floating as oil droplets at the upper layer of the suspension [27].
Table 2. Characteristics of raw POME

| Parameter       | Value          |
|-----------------|----------------|
| COD             | 185,842.13     |
| BOD             | 96,500.00      |
| TSS             | 38,120.00      |
| Oil & Grease    | 59,873.33      |

*Units in mg/L

3.3 Pre-treatment of POME in Batch Test

The POME was treated using 5%, 10% and 15% levels of dilution of garbage enzyme to POME. These were then left for digestion for six days. Parameters such as COD, TSS, and O&G were analyzed. The variations of TSS, COD and O&G with time for different percentages of dilution of garbage enzyme to POME are shown in figures 4, 5 and 6.

Figure 4 shows that TSS was able to be reduced from the beginning until the end of the period of study. TSS in all dilution levels showed a drastic reduction on the sixth day. It is observed that TSS in 10% dilution is significantly the lowest compared to TSS in 5% and 15% dilutions. Lipase applied in wastewater pre-treatment was able to decrease suspended solids and organic matter concentration [28, 29]. Research done by Parmar et al. (2001) also showed that lipase and protease treatment were able to reduce approximately 80% of biosolids in the sewage sludge sample [30].

The reduction of COD is presented in figure 5. It is observed that the concentration of COD reduced from day zero to day six for all 5%, 10%, and 15% level of dilutions. A previous study showed that the reduction of COD was notable only after 27 days of digestion. These might be due to the high initial concentration of COD of garbage enzyme [15]. As mentioned in subsection 3.1, COD is a method used to evaluate the total amount of organic matter. Most of the suspended solids in POME were organic matter [5, 31]. As lipase and protease in the enzyme solution was able to remove TSS, COD can also be reduced.

The outcome of O&G removal is presented in figure 6. It clearly shows that garbage enzyme is able to degrade O&G at different levels of dilution. O&G concentration reduced gradually from day 0 to day 5 for 15% level of dilution. However, it reduced dramatically on day six. A previous study by Jeganathan et al. (2006) indicated that lipase was able to remove O&G in wastewater. Lipases cleave ester bonds of triacylglycerol in O&G during hydrolysis, thus it has the capacity to liberate all types of acyl chains that assist in reducing the concentration of O&G and produce short chain volatile fatty acids (VFA) [32].

Figure 4. Effects of different dilutions of garbage enzyme to POME on TSS
3.4 Efficiency of POME Pre-treatment Using Garbage Enzyme

From figure 7, it is observed that garbage enzyme is very effective in degrading O&G. It was able to reduce about 50%-90% of O&G. The highest reduction percentage was 15% of dilution of garbage enzyme. There is lack of studies reporting on the effectiveness of garbage enzyme in removing O&G. However, Jeganathan et al. (2006) looked at the pet food industrial wastewater treatment with lipase from *C. rugosa* and concluded that the O&G removal was found to be roughly 50% [32].

After six days of treatment, the removal of TSS was in the range of 30%-50% and the removal of COD concentration was approximately between 15%-25%. For the removal of TSS and COD, the most effective level of dilution of garbage enzyme is 10%. This is consistent with the previous study that revealed industrial waste activated sludge treatment with garbage enzyme solution and reported that the TSS removal was found to be roughly 38.6% [1]. According to previous research by Nazim & Meera (2013), garbage enzyme was used to treat greywater and it was found that COD can be reduced within the range of 20%-30% [15].
4. Conclusion
From this study, it was found that the garbage enzyme solution produced was acidic in nature and contained a high amount of organic matter resulting in high COD content. It also contained high amounts of TS, TSS, and TDS. The garbage enzyme solution was also tested for enzyme activity. The results indicate that it possessed amylase, protease, and lipase activity. POME samples that were collected from Chalok Palm Oil Mill were considered to be highly polluted wastewater. Due to the high content of organic material in POME, the COD & BOD values were extremely high. Besides, POME contained high amounts of TSS and O&G. Batch tests were conducted at different dilution levels (5%, 10%, 15%) of garbage enzyme solution to POME. It was able to remove COD, TSS, and O&G. The batch test showed higher percentage of reduction in O&G and lower reduction in COD by garbage enzyme. Garbage enzyme solution with 10% dilution to POME was found to be more effective in removing COD and TSS. For the removal of O&G, 15% dilution of garbage enzyme solution to POME was more effective. In conclusion, POME can be pretreated using garbage enzyme produced from pre-consumer supermarket residues with the efficient removal of stated parameter prior to anaerobic digestion as further treatment. It is suggested that variation of garbage enzyme characteristics with time should be investigated. Suitable additives or activators should be explored to reduce the fermentation period to produce the enzyme.

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