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Temperature and pH Effect on the Aerobic Granules Developed in Palm Oil Mill Effluent

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Abstract: Temperature and pH are crucial in biological wastewater treatment processes. Thus, both of these parameters are studied for their effect on aerobic granules in biological treatment of POME. Three different temperatures (35, 40 and 45°C) and three different pH values (5, 7 and 9) were used for this study. The aerobic granules' performance in terms of COD removal and settling ability was monitored for respective temperatures and pH values for 21 days. It was observed that the COD removal was the highest in 35°C. Up to 85% of the influent COD was removed in the reactor with 35°C. Meanwhile, the highest settling ability (measured in terms of Sludge Volume Index (SVI) value was recorded in the reactor of 35°C. At 35°C, the lowest SVI value (indicates higher settling ability) recorded was 22 mL g^{-1} during the 21 days. In the meantime, the highest COD removal was observed to be 93% in pH 9. In pH 9, the lowest SVI value recorded was 48 mL g^{-1} during the 21 days period. It could be concluded that the aerobic granules could maintain their effectiveness when there is a change of temperature and pH. Furthermore, it could be observed that aerobic granules performed well at average tropical countries' temperature.

Key words: Aerobic granules, temperature, pH, POME

INTRODUCTION

Aerobic granules are a widely researched subject for the past decade. It gained immense attention due to the superior characteristics compared to conventional activated sludge. The aerobic granules are denser than the activated sludge (Pijuan *et al.*, 2009). Thus, it settles better compared to the activated sludge. Moreover, the aerobic granules can sustain its performance under adverse conditions such as shock loadings and toxicity (Arrojo *et al.*, 2004). Besides that, aerobic granules also enable the optimization of land usage for a treatment plant (De Kreuk *et al.*, 2004).

Realizing the potential of the aerobic granules, it was investigated in palm oil mill effluent (POME) (Abdullah *et al.*, 2011; Gobi *et al.*, 2011). The investigation of aerobic granules to treat POME is driven by the current obsolete method of treating POME (series of open pond). Usage of series of open ponds results in poor settling of biomass as well as low removal of COD. In both of the previous study, the aerobic granules were cultivated in POME using sequencing batch reactor (SBR). The SBR was operated at room temperature of 25°C. It took 120 days for the aerobic granules to appear in the study done by Gobi *et al.* (2011) whereas; Abdullah *et al.* (2011) took 17 days only. The major difference between these researchers is the H/D ratio. Higher H/D ratio would enhance the formation of aerobic granules due to the hydraulic attrition and efficient circular vortex from bottom to the top (Qin *et al.*, 2004). Though the aerobic granules appeared at different period of time, the performance of it did not differ much to each other. It was reported that 90 and 91% of the COD was removed from the POME in the study done by Gobi *et al.* (2011) and Abdullah *et al.* (2011), respectively.

Nevertheless, it is highly desirable to obtain similar removal rate of COD at any conditions. It is a common knowledge to understand that pH and temperature have an impact towards the behavior of the microorganisms. Though aerobic granules were successfully developed in our previous studies using POME, the response of aerobic granules towards temperature and pH changes was not demonstrated (Gobi *et al.*, 2011). Hence, the need to study the pH and temperature is critically warranted for POME aerobic granules. At the discharge point of POME, the pH is reported to be around 4.5 to 5.5 (Ahmad *et al.*, 2003). The acidic nature of the POME might affect the microorganisms which forms the crux of the aerobic granules. Meanwhile, the temperature of the POME at discharge point is around 40 to 70 °C (Abdurahman *et al.*, 2011). This range of temperature is considered high for any microorganism activity. Thus, the variation of microorganism activity was studied for aerobic granules.

Till date, no study has been done on the response of developed aerobic granules on temperature and pH changes. However, study has been done on cultivating

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aerobic granules at different temperature and pH. This paper intends to reveal the effect of temperature and pH on the performance of aerobic granules in removing COD and settling ability.

MATERIALS AND METHODS

Preparation of wastewater: Palm Oil Mill Effluent (POME) used in this study was collected from the Elegant Palm Oil Mill, Bagan Serai, Perak, Malaysia. The collected POME was stored in cold storage room at a temperature of 3±2°C. At the collection point, POME normally appears in dark brown color with temperature in the range between 50-60°C. The collected POME normally consists of suspended solids and liquid waste. The POME was filtered to remove the debris and diluted using tap water into desired concentrations before it was fed into SBR. The characteristics of POME used in this study are indicated below.

Aerobic granules: Aerobic granules used for this study were taken from parent SBR reactor. In the parent SBR reactor, aerobic granules were developed using POME as the only substrate. The development of aerobic granules were reported in previous work (Gobi *et al*., 2011).

Reactor setup for temperature and pH study: Six 4 L containers were used to function as reactor. The working volume of the reactors was 3 L with an exchange ratio of 25%. The reactor setup is shown in Fig. 1. Continuous aeration was supplied for the reactor using aquarium pump at the bottom of the reactor. The reactor operates in a 6 h cycle. The reaction cycle consist of 2 min of feeding, 345 min of reaction, 10 min of settling and 3 min of decanting. Three reactors were used for temperature study while the remaining three reactors were used for pH study. All these containers were placed on top of hot plate stirrer which functions to control the temperature as well as to provide stirring inside the reactor. For the temperature study, the temperature of reactors were controlled at 35, 40 and 45°C. The desired temperature for the reactor was adjusted and monitored using a thermometer inside all three containers used. During the temperature study, the pH of the POME was not adjusted. Meanwhile, for the pH study, the pH was controlled using 0.1M HCl and 0.1M NaOH. pH was adjusted only at the beginning of the reaction.

Monitoring the performance of aerobic granule in treating POME: The performance of the aerobic granule in treating POME was closely monitored for COD removal, MLSS concentration, Sludge Volume Index (SVI) and morphology.

Analytical methods used to monitor the parameters

Chemical Oxygen Demand (COD) removal: The COD analysis was carried out using the COD vials (1 vial per sample). In these COD vials, chemicals ($K_2Cr_2O_7$, $AgNO_3$, $HgSO_4$, Potassium hydrogen phthalate, H_2SO_4) were pre-mixed. Two milliliters of sample was filtered using a syringe filter (MF-Millipore Millex GS syringe filter with pore size 0.22 μm) and inserted into the prepared vials. A blank sample was prepared in a different vial by inserting 2 mL of distilled water. The COD vials were left to digest at 150°C for 2 h in a digester. After 2 h, the vials were cooled down to room temperature and a COD kit (Checkit Direct, Lovibond) was used to measure the COD value in each vial.

MLSS concentration: The MLSS concentration of the sample was measured according to the standard method APHA, AWWA and WEF2005 Standard Methods for the examination for Water and Wastewater, 21st Ed. APHA, Washington DC, USA. Liquid sample was mixed

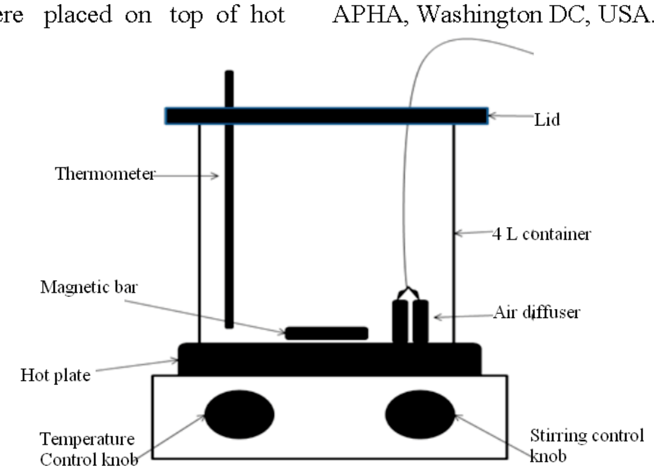

Fig. 1: Reactor setup for both temperature and pH study

with a magnetic stirrer in order to obtain a uniform and homogeneous sample. While stirring, 10 mL of the sample was withdrawn and filtered on a Whatman GF/C filter paper (1.2 μm pore size) using a vacuum filter. A filter paper was carefully washed with distilled water. Then, the filter paper was dried at least 1 h at 103 to 105°C in a drying oven (Memmert), cooled in a desiccator to balance the temperature and weighed. The cycle of drying, cooling and weighing was repeated until a constant weight was obtained. The measurement was carried out in triplicate and an average value was calculated. The MLSS value was calculated using Eq. 1 below:

$$\text{MLSS,mgL}^{-1} = \frac{(A - B)}{(\text{Sample volume,mL})} \times 1000 \quad (1)$$

where, A is the weight of sample and filter paper (mg) and B is the weight of empty filter paper (mg).

Sludge Volume Index (SVI): The Sludge Volume Index (SVI) was carried out according to the standard method APHA, AWWA and WEF2005Standard Methods for the examination for Water and Wastewater, 21st Ed. APHA, Washington DC, USA. One litre of mixed liquor with suspended solids was withdrawn from the reactors and inserted in a one litre measuring cylinder. After 30 min, the volume of settled granules (or sludge) in the measuring cylinder was recorded. The value was divided with the MLSS value of sample and multiplied by 1000 to obtain the SVI value in the unit of mL g^{-1}.

Morphology of aerobic granule: The morphology of the aerobic granules was observed using microscope. In this research, a trinocular stereozoom microscope (Olympus SZX9) was used to observe the granule's surface morphology using magnification in between 6 and 50. In this observation, the surface structure of the aerobic granule was able to be seen.

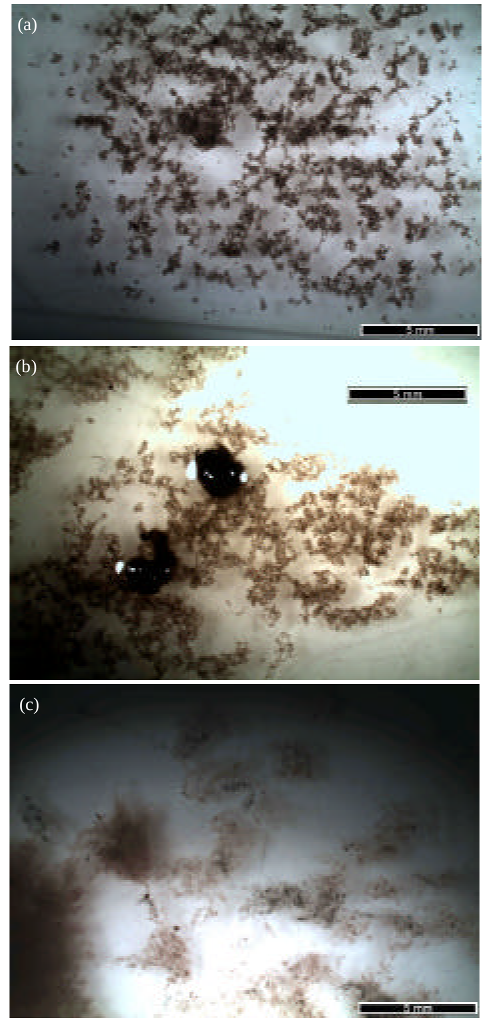

Fig. 2(a-c): Morphology of aerobic granules at (a) 35, (b) 40 and (c) 45°C

RESULTS AND DISCUSSION

Effects of temperature on aerobic granules morphology and performance: The aerobic granules were studied for their effect at temperatures of 35, 40 and 45°C. The chosen temperatures falls in the typical temperature range of the POME coming into the cooling pond at the conventional biological treatment system. It has been reported in the previous work that the temperature has an effect on the morphology and the performance of the aerobic granules (Song *et al*., 2009). However, the works on analyzing the effect of temperature on aerobic granules is still lacking.

Figure 2 shows the morphology of the aerobic granules at various temperatures. Figure 2 demonstrates that the shape of the aerobic granules at all temperatures became irregular and very few aerobic granules were spotted as the temperature increased. However, there are more aerobic granules at 40 than 35°C. However, the aerobic granules at 45°C completely lost shape or in other words disintegrated from its compact structure. This phenomenon could be due to the breakage of matrix that holds the aerobic granules in shape at higher temperature. This eventually led to difficulties in determining the average granules size. Furthermore, the disintegrated aerobic granules at 45°C have major impact on the performance of the aerobic granules in removing COD.

Figure 3 shows the effect of temperature on the COD removal percentage. From the Fig. 3, it can be observed that the temperature has a major impact on the performance of the aerobic granules in treating the POME. The lowest temperature (35°C) demonstrates the best performance (85% removal at average) in terms of

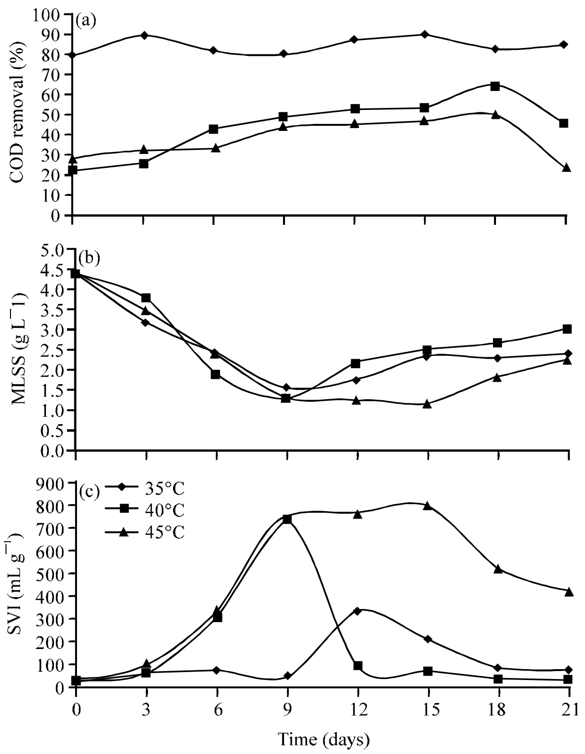

Fig. 3: Effect of temperature on (a) COD removal percentage, (b) MLSS concentration and (c) SVI value of aerobic granules

removed COD. Meanwhile, the highest temperature (45°C) shows the lowest performance (in between 20 to 30%) of the aerobic granules to remove COD. Even though there had been increase in the COD removal percentage for temperatures of 40°C and 45°C as a function of time, the performance of the aerobic granules were not stable and eventually dropped on the 21st day.

At higher temperature, the activity of the aerobic granules was lower due to the denaturing of the enzymes in the microorganisms. Hence, the aerobic granules could not oxidize the COD available in the reactor. Moreover, the main SBR (parent reactor) was operated at room temperature and the COD removal performance of the reactor was much higher (90% at average) compared to all the tested temperatures.

Apart from that, the MLSS concentration of the reactor and the SVI value of the aerobic granules show fluctuating results throughout the experiment period. During the initial days of the reactor operation, similar trend can be observed in terms of reduction of MLSS concentration in all the reactors. However, it could be observed that the MLSS concentration at 40°C reduced the least and later increased to the highest value (3 g L^{-1}) among the all the temperatures. Meanwhile, the MLSS concentration at 45°C reduced to almost 1.5 g L^{-1} and it increased to around 2 g L^{-1} only on the 21st day. Besides that, the SVI value increased and settling ability of the biomass in the reactor has reduced drastically at all the three temperatures over the period of 21 days. The aerobic granules in 45°C recorded the highest SVI value on 15th day (800 mL g^{-1}).

Table 1: Aerobic granules performance and morphology at different temperatures

Temperature (°C)	Average COD removal (%)	Morphology
35	85	Very few AG present
40	35	AG present
45	25	No AG present

The decrease in aerobic granules concentration and the increasing SVI value could be correlated to the morphology of the aerobic granules. As a function of time, the aerobic granules in all the temperatures almost disintegrated (as it is evident in Fig. 2) and subsequently leading to poor settling ability. The poor settling ability has contributed to the decrease in MLSS concentration and increase in SVI value. Table 1 shows the summary of aerobic granules' performance and morphology at three different temperatures.

Effects of pH on the aerobic granules morphology and performance: The pH values of the POME had an effect on the aerobic granules morphology. Figure 4 shows the effect of pH on the aerobic granules morphology. When the aerobic granules were seen under the microscope, it can be observed that in pH 9, the darker colour aerobic granules were dominating in the reactor. Meanwhile, in pH 5 and 7, the colour of the aerobic granule was lighter. Moreover, the size of the aerobic granules also varies notably as the pH increased. The aerobic granules in pH 9 have the highest diameter of 2.832 mm while the aerobic granules in pH 5 have the smallest diameter (1.302 mm). The colour of the aerobic granules could be related to the maturity of the aerobic granules. The darker aerobic granules are more matured compared to the lighter ones.

It has been reported in the earlier works that the darker aerobic granules exhibits compact granules compared to the yellow-brownish granules (Li *et al*., 2010). It has been further proved in SVI value where the aerobic granules in pH 9 can settle considerably well compared to the other pH medium. Similar observation has been made in previous work (Li *et al*., 2010), where the elevation in pH has resulted in formation of compact granules, though the source of wastewater is different from this study.

Meanwhile, the pH of the POME also had an effect on the performance of the aerobic granules. Figure 5 (a)

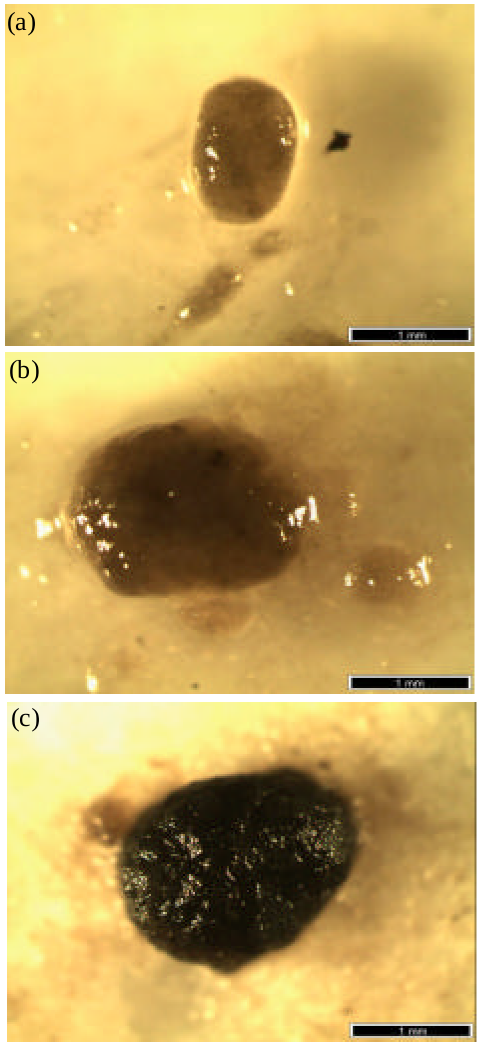

Fig. 4(a-c): Morphology of aerobic granules at (a) pH 5, (b) pH and (c) pH 9

shows the COD removal of the aerobic granules in the POME at different pH values. From the Fig. 5 a, the obvious difference of performance was seen during initial period of the reactor operation, where the POME in pH 9 has highest COD removal percentage (93%) on the 3rd day. Moreover, the COD removal percentage was seen to be lowest (77%) for the pH of 7. However, for all the pH values, the COD removal percentage became stable and achieves almost similar values on the 21st day. The highest COD removal recorded was around 95% at pH 7 on 15th day.

pH values also had an effect on the concentration of the aerobic granules. Figure 5 (b) shows the effect of pH on the MLSS concentration. From the Fig. 5 b, it can be deduced that in all pH values, the concentration of the

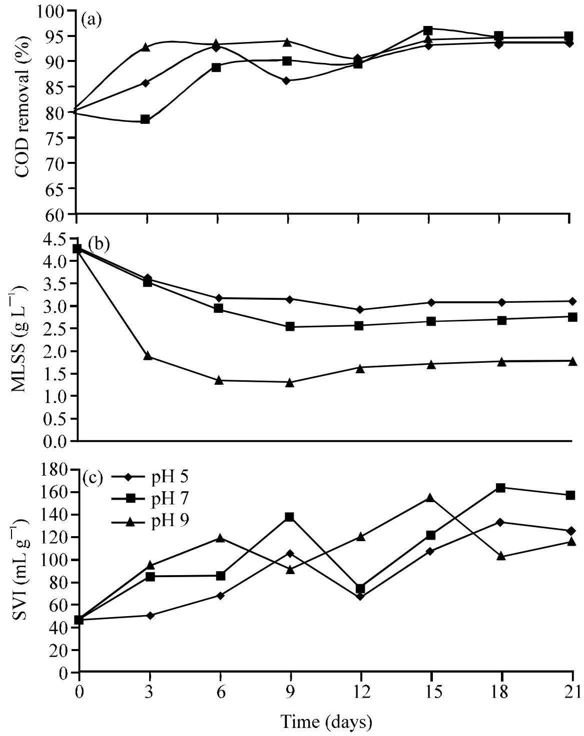

Fig. 5(a-c): Effect of pH on (a) COD removal percentage, (b) MLSS concentration and (c) SVI value of aerobic granules

Table 2: Aerobic granules performance and characteristics at different pH

pH	Average COD removal (%)	Morphology	Average size (mm)
5	87	Brown AG	1.3
7	85	Brown AG	1.8
9	90	Dark AG	1.9

aerobic granules reduced compared to the 1st day. However, pH 9 had the least effect on the decrease of aerobic granules concentration. Meanwhile, the concentration of aerobic granules in pH 5 reduced the most (from 4.4 to 1.9 g L^{-1}) compared to other pH values.

The reduced concentration of the aerobic granules has effect on the SVI value as well. Figure 5 (c) shows the effect of the pH on the SVI values. From the Fig. 5 c, it can be observed that the SVI values were fluctuating and show an increasing trend throughout the operation of the reactor. Nevertheless, the SVI values of pH 9 shows lower value compared to pH 7 on 21st day. It can be closely related to the minimum loss of aerobic granules concentration as discussed earlier. A lower SVI value indicates that the aerobic granules are able to settle better. It has been further proved in the aerobic granules morphology, where the aerobic granules in pH 9 has more compact and round structure compared to the aerobic granules in pH 5 and 7. Table 2 shows the summary of aerobic granules performance at three different pH.

CONCLUSION

It is evident from the study that the temperature and pH had an effect towards the aerobic granules activity. It is shown that at 35°C, the COD removal was the highest (85% removal). However, the MLSS and the SVI value did not yield desired results. Meanwhile, in pH 7, the COD removal percentage was the highest (95% removal). Nevertheless, the MLSS and SVI value were not promising. In a nutshell, it can be concluded that aerobic granules morphology and performance varies in relative to changes in pH and temperature. Though there is a variation, the performance of the aerobic granules was in the acceptable range.

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