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Effect of Biodegradation on the Physical Properties of Palm Oil Mill Effluent (Pome) using Mixed Culture of Fungi

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This study investigated the effects of biodegradation by the mixed culture of fungi (*Pithomyces sacchari* and *Pestalotiopsis maculans*) on selected physical properties of palm oil mill effluent (POME). Mixed culture innoculum (4% v/v) was added to autoclaved and raw POME samples, which were subjected to biodegradation at 120rpm and 35 ºC for six days. The pH, electrical conductivity, total dissolve solids and biosolids of the digested samples were quantified at 24 h intervals. These parameters for the autoclaved sample, at the end of the digestion period, were 6.88, 4.38mS/cm, 2.28 g/L and 25.6 g/L, respectively. These values were higher than 6.34, 4.24mS/cm, 2.22 g/L and 22.87g/L obtained for the raw POME sample, respectively. The kinetic studies of the degradation of POME, based on the concentration of the biosolids, were also investigated. The kinetic studies show that the degradation of the raw POME sample best fits the zero order kinetic model (R^2 = 0.96), while the degradation of the autoclaved POME sample best fits the first order kinetic model (R^2 = 0.83). However, the digested POME may require further treatment in order to meet standard suitable for discharge into the water body.

Key words: Biodegradation, Mixed culture, Zero order, First order, *Pithomyces sacchari, Pestalotiopsis maculans*.

Palm oil mill effluent (POME) is typical wastewater originating from the mixture of sterilizer condensate, separator sludge and hydrocyclone wastewater in palm oil mill industry. The rapid expansion in the palm oil mill industry has led to the increase in the volume of POME injected in the environment[1,2]. POME has been categorized as high strength wastewater due to its high biochemical oxygen demand (BOD) which is about 100 times the BOD of polluting domestic sewage[3]. Although no chemical was added to the palm oil extraction process, yet the resulting POME is characteristically acidic (pH≤4.5) and this may be attributed to the complex organic acids present in the POME[4,5,6]. Fresh POME is usually a hot and acidic brownish colloidal suspension, characterized by high amounts of total solids, oil and grease, chemical oxygen demand (COD) and biochemical oxygen demand (BOD) and the concentrations vary depending on the source[2]. The presence of high percentage (50%) of particulates has contributed to the organic loading of the POME, thus rendering it unsafe for discharge into the environment unless treated[3].

Large volume of POME, generated from the increasing palm oil industries in countries such as Malaysia which produce over 53 million m^3 per year, cannot be accommodated any longer in the host environment[7,8]. The discharges of untreated or partially treated wastewater poses significant environmental impacts on freshwater biota[9]. The presence of high organic matter in POME has made it susceptible to biological treatments based on anaerobic, aerobic and facultative processes, since

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these organic matters are generally biodegradable[10]. The biological treatment is facilitated by the activities of the consortium of active microorganisms, which utilize the organic substances present in the POME as nutrients and consequently led to loss in the pollutant load present in the POME[4,11,12]. The application of microorganisms, such as fungi, bacteria and yeast for the treatment of POME have been reported by various authors such as Karim and Kamil[13], Bhumibhamon *et al*.[14], Oswal *et al*.[15], Najafpour *et al*.[16], and Vijayaraghavan *et al*.[17]. However, this study investigates the degradation of POME with mixed culture of fungi (*Pithomycessacchari* and *Pestalotiopsis maculans*), isolated from sludge palm oil mill (SPO). Basically the effect of the biodegradation by the mixed culture on the physical properties of the POME was analyzed and reported.

MATERIALS AND METHODS

Materials

Palm oil mill effluents and sludge palm oil (SPO) samples were obtained at the West Palm Oil Mill, Carey Island, Selangor, Malaysia. The samples were later stored at 4 ºC, before use, at the Bioenvironmental Engineering Laboratory of International Islamic University Malaysia (IIUM) Malaysia. Analytical grades reagents were used in this experiment throughout. All meters and equipment were checked and calibrated according to the manufacturer's specifications.

Inoculum

The two pure samples of the fungi (*Pithomyces sacchari* and *Pestalotiopsis maculans*) used in this study were isolated from sludge palm oil (SPO), at Bioenvironmental Laboratory of Biotechnology Engineering Department, IIUM, Malaysia, and reported in previous studies. The pure sample of each fungus was maintained on potato dextrose agar (PDA) plate, separately, and incubated at 22± 2ºC, for 7 days[18]. The mature fungi were subjected to three transfers, at least, on PDA before being used for inoculation[18]. The innoculum of 7–day culture of each fungus in spore form (1 x 10^5 conidial/mL) was obtained by washing the mature fungi with sterile distilled water. The mixed culture was developed by mixing the inoculum of the two fungi in the ratio 1:1 (v/v).

Biodegradation Test

Batch biodegradation test was carried out in 250ml Erlenmeyer flasks filled with 100ml of the homogeneous POME sample. The POME sample was diluted with distilled water (1:1, v/v), stirred thoroughly for 30min and thereafter divided into two parts. A portion was used directly without further process and henceforth referred to as 'Raw POME Sample' in this study. The other portion was autoclaved at 121ºC for 15 min and referred to as 'Autoclaved POME Sample'. 100mL of each homogenous POME samples was inoculated with required concentrations (4% v/v,) of the mixed culture (1:1 v/v) aseptically. Thereafter the mixture of the POME and innoculum was agitated at 120 rpm and 30 ºC temperature for 7days [19].

Determination of Biosolids

The total mass generated after digestion is referred to as the biosolids, which was obtained as the residues retained on the Whatman filter paper [20]. The filter paper was dried to constant weight at 105 ºC, stored in the desiccators to cool and subsequently weight (W_1). It was then placed on a funnel, over which 100 mL of sample was poured and left for 5h, after which the filter paper and the residue were dried in the oven at 105 ºC overnight. The final weighed (W_2) was determined and the mass of the biosolids was quantified according to the expression in equation 1. The experiment was conducted in triplicates.

$$Biosoilds = \frac{(W_2 - W_1)}{V} \quad ...(1)$$

[W_1=weight of filter paper, W_2=weight of filter paper and residue, V=volume of sample]

Determination of pH, TDS, and EC

The pH was measured with a glass electrode pH Meter (senSion5 pH, Switzerland) dipped into the digested samples. Similarly, total dissolved solids (TDS), and electrical conductivity (EC) of the digested samples were measured with Microprocessor Conductivity Meter (HACH senSion5, USA). All readings were repeated in triplicates and the average was used for further analysis.

RESULTS AND DISCUSSION

The physical and chemical properties of the POME sample are expected to be affected by

biodegradation test particularly of the POME sample with mixed culture of fungi. The changes in the selected physical properties of the raw and autoclaved POME samples were observed and compared.

Effects of Combined Factors on Total Dissolved Solids (TDS) in POME Sample

Total dissolved solid (TDS) is one of the important physical properties which indicate the presence of soluble inorganic salts in the wastewater [21]. Similarly, it is a convenient measure of the total ionic concentration in water [22]. Large amount of TDS may lead to increased mineralization of the receiving water body and consequently deplete dissolved oxygen of such water body. McCulloch *et al.*,[23] noted that elevated TDS concentration can cause osmotic stress which affects the osmoregulatory capability of the organisms, particularly, freshwater animals. Average concentration range of TDS for typical POME sample is given as 15.5 – 29g/L [3]. In this study, the initial TDS concentration of the POME sample was 2.5g/L and this dropped sharply for the first three days to 2.18g/L for the sterilized POME sample, but increases slightly to 2.28g/L on the sixth day (Figure 1).

The concentration of the TDS, in the raw POME sample dropped gradually to 2.18g/L on Day 5 and then rose to 2.22g/L on Day 6. Generally, the decrease in the TDS concentration for raw POME was higher than those of corresponding sterilized POME samples at each day, except on the Day 3. This may suggest that other indigenous microorganism may have competed with the mixed culture introduced into the raw POME sample thereby causing high leaching of the dissolved solids unlike in the case of the autoclaved POME sample. Furthermore, the close similarity in the decrease of TDS concentration in both POME samples justifies that the mixed culture introduced are effective in biodegradation of POME.

Effects of Combined Factors on Conductivity in POME Sample

Conductivity is a measure of the total amount of ions present in a body of water and can be used to estimate chemical richness of a water body. Acceptable limits of 70 µS/cm and 250 µS/cm have been reported for the conductivity in domestic water supply and discharged into the receiving water bodies, respectively[24]. The conductivity of the autoclaved POME sample decreased from

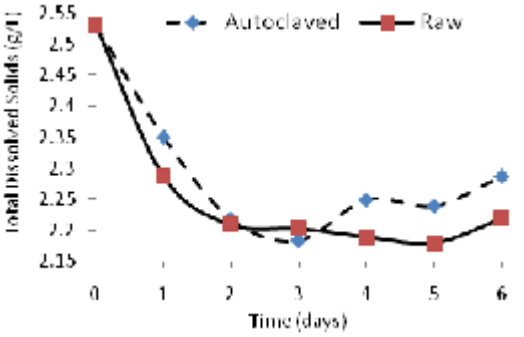

Fig. 1. Effect of combined factors (34 ºC, 130rpm and 4 % v/v mixed culture) on the total dissolved solids of POME sample

initial conductivity of 4.82 mS/cm to 4.20 mS/cm on Day 3 but increased afterwards to 4.38mS/cm. The conductivity of the raw POME sample decreased to 4.19mS/cm on Day 5 and then increased to 4.24 mS/cm on Day 6, respectively, (Fig. 2). The reduction in the conductivity of the autoclaved POME sample was generally higher than those obtained for the corresponding raw POME sample, except on Day 3, though the conductivity increased afterwards to 4.38 and 4.24 mS/cm for the autoclaved and raw POME samples, respectively, on the sixth day. The trend in the decrease of conductivity is slightly close for the autoclaved and raw POME samples under the biodegradation with mixed culture of *P. sacchari* and *P. maculans*, and this further substantiates the effectiveness of the fungi in the biodegradation of POME.

Effects of Combined Factors on pH in POME Sample

The initial pH (4.48) of the POME samples used in this study was slightly higher than the average range (4.15 to 4.45) reported in the literature[3]. The pH of the digested autoclaved POME sample increased from the slightly acidic region (4.48) to neutral region of 7.21 on Day 3, but dropped slightly to 6.88 on Day 6 (Figure 3) and this may be considered safe for discharge according to DOE Standard[25]. Similarly, the pH of the raw POME sample also increased to its peak (6.6) on Day 2 and dropped to 6.34 on Day 6. Comparatively, the pH of the autoclaved POME sample was higher than those obtained for the corresponding raw POME sample. The pH values obtained in this study fall within the water quality ranges (pH 6.5 to 8.5) intended for full contact recreation[25], but

slightly less than the World Health Organization standard (pH 7.0 to 8.5) acceptable for water drinking water[26,27]. Similarly, the pH values obtained for the treated POME samples falls within the European Union pH limits (6.0 to 9.0), sets for fisheries and aquatic life[28] and this is considered favorable, since the treated POME samples is targeted for discharge into aquatic environment.

Effects of Combined Factors on Biosolids in POME sample

POME naturally contains relatively high solids in the range of 33,790 - 37,230mg/L as total solids[3]. The term biosolid is however used in this study to include all solids that can be retained on filter paper after drying, thus it includes the cells of the microorganisms and other solid particles that might have remained in the digested POME samples[3]. Figure 4 shows the reduction of biosolids in the digested POME samples by the mixed culture of *P. sacchari* and *P. maculans*. There was an increase in the biosolid contents in the autoclaved POME samples, within the first 24 h. It is suspected that, since other indigenous microorganisms present in the POME have been deactivated through the sterilization process, the mixed cultures of *P. sacchari*and *P. maculans* might have used all the necessary nutrients in the media for growth of the body mass thereby increasing the overall biomass present in the POME[29].

The concentration of biosolids, present in the autoclaved POME sample, reduced from 31.4g/L to 25.6g/L as the biodegradation continued until the sixth day. The concentration of biosolids, present in the raw POME sample, decreased from 26.20g/L to 22.87g/L on Day 6. This development further supports the fact that the mixed culture of *P. sacchari*and *P. maculans* influenced the biodegradation of the autoclaved and raw POME samples by using all the necessary nutrients and solids for effective metabolism. The degradation might be used to study the kinetics of degradation of the POME[19].

Kinetic Study

The kinetic of the degradation of the POME sample, based on the selected physical properties, was investigated for the zero and first

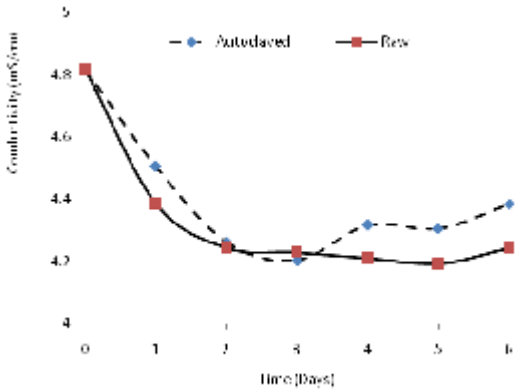

Fig. 2. Effect of combined factors (34 ºC, 130rpm and 4 % v/v mixed culture) on the conductivity of POME sample

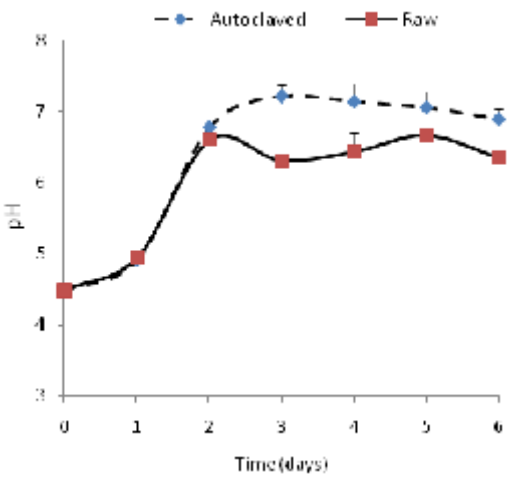

Fig. 3. Effect of combined factors (34 ºC, 130rpm and 4 % v/v mixed culture) on the pH of raw and autoclaved POME samples

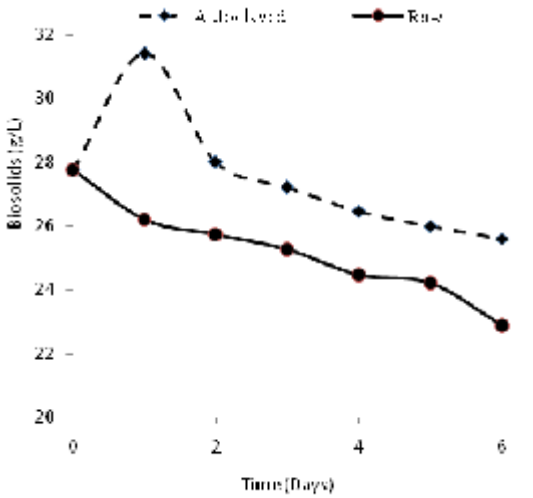

Fig. 4. Effect of combined factors (34 ºC, 130rpm and 4 % v/v mixed culture) on the biosolids content of POME sample

order kinetics. The degradation of the biomass was selected as reference since this relates more to the mass concentrations of the POME[19]. Similarly, the biosolids contain the substrates that the mixed culture of fungi degraded in the media. The zero order and first order kinetic models are stated in equations 2 and 3 respectively. The zero order was evaluated by plotting 'S' against 't', (Fig. 5) and the first order was evaluated by plotting'lnS' against 't', (Fig. 6). The constants for all the models were evaluated.

$$S_f = S_o - k_0 t \quad ...(2)$$

$$InS_f = InS_o - k_1 t \quad ...(2)$$

where 'S'_f ($kgmolm^{-3}.min^{-1}$) is the volumetric rate of reaction, 'S_0' ($kgmolm^{-3}$) is the concentration of the substrate; 'K_0' is the zero-order rate constant ($molm^{-3}.min^{-1}$); 'K_1' is the first-order rate constant (min^{-1}), 't' is time (min).

The coefficient of regression (R^2), 0.9586 and 0.9508, of the zero and first order kinetic models, respectively, for the raw samples are higher

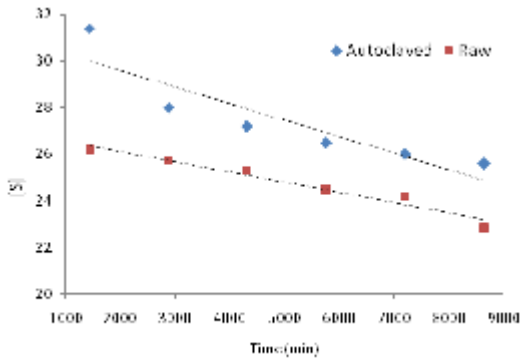

Fig. 5. Zero order kinetic model for the biodegradation of POME samples by mixed cultures of fungus

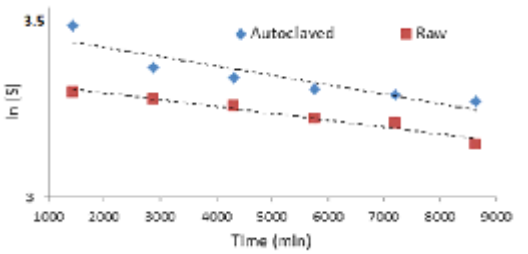

Fig. 6. First order kinetic model for the biodegradation of POME samples by mixed cultures of fungus

than R^2 (0.8122 and 0.8341) obtained for the corresponding autoclaved samples. This suggests that the degradation of the raw POME sample by the mixed culture best fits the zero order kinetic model ($R^2 = 0.9586$), while the degradation of the autoclaved POME best fits the first order kinetic model ($R^2 = 0.8341$). In comparison, the zero and first order rate constants, $k_0 = 0.7 mgl^{-1}min^{-1}$ and $K_1 = 0.03\ min^{-1}$, respectively, while the values obtained for the degradation of the autoclaved POME sample were = $0.4\ mgl^{-1}min^{-1}$ and = $0.02\ min^{-1}$, respectively.

Conclusively, the degradation of palm oil mill effluent (POME) sample with mixed culture of fungi (*P. saccharia*nd *P. maculans*) has shown significant effect on the physical properties of the digested raw and autoclaved POME samples. The selected physical properties such as pH, electrical conductivity, total dissolve solids and biosolids of the POME samples were reduced as the biodegradation time increased. However, this may be considered as pre-treatment for further POME treatment procedure, such as anaerobic, in order to improve the qualities of the POME to meet standard suitable for discharge into the water body. The kinetic studies demonstrate that the degradation of the raw POME sample best fits the zero order kinetic model. Further studies would investigate the effect of the biodegradation on the biochemical properties of the biodegraded POME sample.

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