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Biofilm-coated macrocomposites for the treatment of high strength agricultural wastewater

Mazidatul Ashiqeen Balqiah Mohamad Lazim[a], Chin Hong Neoh[b], Chi Kim Lim[a], Chun Shiong Chong[c], Zaharah Ibrahim[a,*]

[a]*Faculty of Biosciences and Medical Engineering, Department of Biosciences and Health Sciences, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia, Tel. +60 176968380; email: mashiqeen@gmail.com (M.A.B. Mohamad Lazim), Tel. +60 177692501; email: cklim1986@yahoo.com (C.K. Lim), Tel. +60 75557545; Fax: +60 75566162; email: zaharah@fbb.utm.my (Z. Ibrahim)*
[b]*Institute of Environmental and Water Resources Management, Water Research Alliance, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia, Tel. +60 124886775; email: chinhong@hotmail.com*
[c]*Faculty of Biosciences and Medical Engineering, Department of Biotechnology and Medical Engineering, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia, Tel. +60 755 57554; email: cschong@utm.my*

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ABSTRACT

The processing of palm oil generates highly polluting effluent called palm oil mill effluent (POME). Due to the inefficiency of conventional treatment methods to meet the environmental standard discharge limit, alternative treatment methods could play a positive role in treating POME. In this study, biofilm-coated macrocomposites were used to treat the POME obtained from palm oil industry. Color and chemical oxygen demand (COD) removal were the main parameters analyzed in this study. The rate of color removal was examined using the Elovich equation, Pseudo-first-order, and Pseudo-second-order kinetics models. Pseudo-first-order gave the best model equation for color adsorption of POME. The biofilm that was coated on the macrocomposites consisted of a mixed bacterial culture of *Brevibacillus panacihumi*, *Enterococcus faecalis*, *Lysinibacillus fusiformis*, and the newly identified *Klebsiella pneumonia* (MABZ). All of these bacteria were previously confirmed as bacteria capable of removing color and COD. During the treatment, results showed color reduction of POME up to 92% (initial 3514 ADMI) and COD removal of 93% (initial 888 mg/L) after 6 d of incubation. The color removal and COD reduction of the final POME were enhanced by biofilm-coated macrocomposites.

Keywords: Palm oil mill effluent; Decolorization; Biofilm; Adsorption; Pseudo-first-order kinetics

1. Introduction

During the production of palm oil, high organic strength of effluent called palm oil mill effluent (POME) is generated. It was estimated that about 2.5 tonnes of POME is generated for every tonne of crude palm oil produced [1]. The effluent is highly colored primarily due to lignin and its degraded products, tannin and humic substances from the crushed palm nut besides the lipids and fatty acids from incomplete steam extraction [2]. Although a large number of palm

*Corresponding author.

oil mills use biological ponding system as their conventional treatment of POME, these systems, however, do not remove color from effluents effectively [3]. It should be highlighted that the POME in the conventional ponding system is treated without the addition of chemicals and is dependent solely on the presence of indigenous microorganisms. Treatment of POME using ponding systems could not cope with the high chemical oxygen demand (COD) load of wastewater released by the mill and fails to meet the standard discharge limit.

Various studies for the treatment of POME such as membrane technology [4], electrocoagulation [5], coagulation–flocculation [6], and decolorization by living fungus [3,7] have been conducted. However, combination of physical and biological treatment using biofilm-coated macrocomposites have been the current interest in terms of lower initial cost and ease of operation [8]. Zeolite-activated carbon macrocomposites are effective in adsorption of dye Acid Orange 7 [8] and petrochemical wastewater [9]. However, macrocomposites have never been used in the treatment of high-strength organic wastewater such as POME.

This study was conducted to improve the current treatment system of color and COD removal by integrating both physical and biological method for the treatment of POME. Three different kinetic models (pseudo-first-order, pseudo-second-order, and Elovic equation) for the adsorption of color from POME were also presented.

2. Materials and methods

2.1. Sample collection and preservation

Final POME was obtained from a local mill factory in Johor, Malaysia. The wastewater was stored at 4°C prior to further use.

2.2. Microorganism and identification

Brevibacillus panacihumi, *Enterococcus faecalis*, and *Lysinibacillus fusiformis* were obtained from the Faculty Biosciences and Medical Engineering that have the ability to treat dye Acid Orange 7 [10] and textile wastewater [11]. Bacteria isolated from anaerobic POME using the dilution streak plate methods were also used to treat final POME. To identify the bacterium MABZ, genomic DNA was extracted from an overnight culture in nutrient broth. Isolation of genomic DNA was done according to the protocols provided by Promega (Wizard® Genomic DNA Purification Kit). PCR amplification was then carried out to amplify DNA using the universal primer pA (5′- AGA GTT TGA TCC TGG CTC AG-3′) and primer pH (5′-AAG GAG GTG ATC CAG CCG CA-3′) before the DNA was sent for sequencing. After that, multiple sequence alignment was carried out using ClustalW while phylogenetic tree was constructed using neighbor-joining distance method in the MEGA4 software.

2.3. Development of macrocomposites

Table 1 shows the components of macrocomposites used in this study. Water was added gradually into the mixture until all the components were well mixed. The mixture was placed into polyethylene mold to form cubes and the cubes were left to solidify. The mold was covered with aluminum foil to prevent excessive loss of water by evaporation. After that, the macrocomposites formed were demoulded and cured in water for 48 h. Finally, the macrocomposites were air-cured for another 48 h.

2.4. Biofilm formation on macrocomposites

The three bacteria were grown individually and were mixed in ratio of 1:1:1 (v/v). The mixed bacterial culture (20% v/v) was transferred into sterilized wastewater (200 mL) containing 10% (v/v) nutrient broth. The macrocomposites (50%, v/v) were transferred into flasks and incubated at 150 rpm, 37°C for 6 d. Then the macrocomposites were taken out and dried at 105°C to constant weight. The macrocomposites were then viewed using the Field Emission Scanning Electron Microscope (FESEM).

2.5. Kinetic study of color sorption in POME

Kinetic study was carried out in a 500 mL beaker containing 50% w/v macrocomposites. Aeration was supplied using the air pump. The rate of color sorption was measured at various time intervals up to 5 h.

Table 1
Components and percentage for development of macrocomposites

Material	Percentage (%)
Aggregates	70
Sand	10
Ordinary portland cement	10
Activated carbon	5
Zeolite	5

2.6. Treatment of final discharge POME

Four different conditions were set up for the treatment of POME; (i) mixed bacterial culture (suspended cells, 20% v/v), (ii) macrocomposites without bacteria (50% w/v), (iii) biofilm-coated macrocomposites, and (iv) without bacteria and macrocomposite. All of these were prepared in triplicates. The flasks were incubated at 37˚C, 150 rpm for 6 d. The color and COD were taken at regular time intervals.

2.7. Analytical methods

At the end of the experiments, all of the samples collected were centrifuged at 4,000 rpm for 15 min at 4˚C. The supernatant was used for determination of color (ADMI, American Dye Manufacturing Index, unit) and COD. The pH was measured using the Sartorius PB-10 pH meter. All experiments were performed in triplicates. All results were expressed as mean ± standard error of three replicates. The statistical analyses were performed using SPSS Statistics 17.0.

3. Results and discussion

3.1. Mixed bacterial culture for treatment of color and COD

The strain MABZ was identified using the 16S RNA gene analysis. The phylogenetic tree was then constructed to determine the evolutionary relationship between the groups of organisms. Strain MABZ shared more recent ancestors in a cluster with *Klebsiella pneumoniae* (MABZ) with the bootstrap values of 100% (Fig. 1). Besides the newly identified bacteria MABZ, three of the previously isolated bacteria (*B. panacihumi*, *E. faecalis*, and *L. fusiformis*) showed abilities to reduce color and COD. They were subsequently used as a mixed culture for biofilm formation.

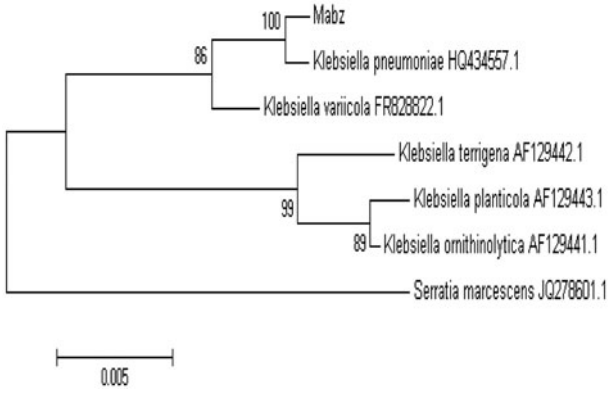

Fig. 1. Phylogenetic tree illustrating the relationship between MABZ and its related species.

3.2. Biofilm formation on macrocomposites

Figs. 2 and 3 are micrographs of FESEM which illustrate the surfaces of a macrocomposite without biofilm (control) and macrocomposites immobilization with the mixed bacterial culture of *E. faecalis*, *B. panacihumi*, *L. fusiformis*, and *K. pneumonia* (MABZ) during the treatment of POME, respectively. In Fig. 2, the spiky structures were probably due to the formation of calcium silicate hydrate (C–S–H) gel. During cement hydration process, lime is produced and it reacts with alumina silicate component of zeolite. Interactions between the ions cause the formation of C–S–H gel. The C–S–H gel is a disordered material composed of short silicate chains held together by calcium oxide regions, and with water trapped inside the structure [12]. This gel is the main cement strength based material that provides stability, and increases density, hardness, and durability, No. C–S–H gel was found on the macrocomposites. In Fig. 3, bacteria of different sizes ranging from 1.3 to 1.8 μm were found on the surfaces of macrocomposites. Bacterial Extracellular polymeric substances (Fig. 4) which are responsible for attachment onto surfaces were also observed.

3.3. Kinetic study of color sorption in POME

The mechanism of adsorption process was examined using three simplified models: Pseudo-first-order, Pseudo-second-order, and Elovich model. The Pseudo-first-order model is as follows [13]:

$$\log(q_e - q_t) = \log q_e - k_1 t \quad (1)$$

where q_e and q_t are the amount of color adsorbed (mg/g) at equilibrium and at time t, respectively, and k_1 is the rate constant of sorption (L/min). The

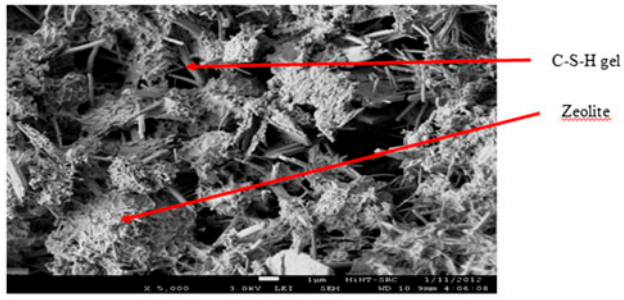

Fig. 2. FESEM micrograph of macrocomposites (control).

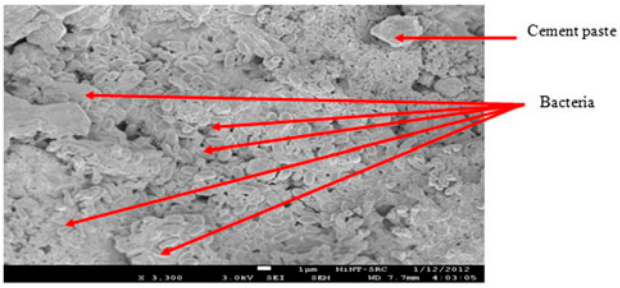

Fig. 3. FESEM micrographs of macrocomposites with biofilm.

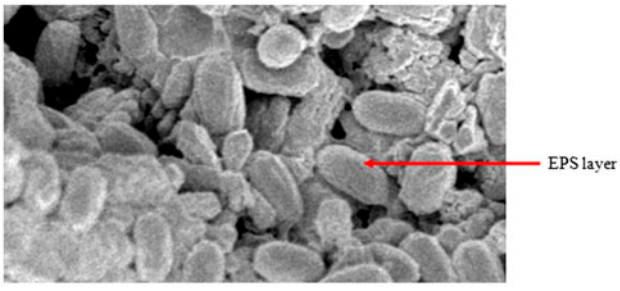

Fig. 4. Extracellular polymeric substances formation by biofilm.

Pseudo-first-order plot of color adsorption onto macrocomposite is shown in Fig. 5. Values of k_1 and q_e can be obtained from the slope and intercept of the plot. The R^2 value of this model, 0.934, showed a good agreement between the experimental and the calculated q_e values.

The Pseudo-second-order equation is expressed as:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \tag{2}$$

where k_2 (g/mgt) is the rate constant, and q_e can be determined from slope and intercept of linear relationship of plot t/q_t vs. t, as shown in Fig. 5. For this plot, the R^2 value was 0.890.

Elovich equation is generally expressed as:

$$\frac{1}{q_t} = \frac{\ln(\alpha\beta)}{\beta} + \frac{\ln t}{\beta} q \tag{3}$$

where α and β can be determine from the slope and intercept of linearly correlated plot q_t vs. t. The R^2 for this equation was 0.904 as shown in Fig. 5.

Results showed that the pseudo-first-order kinetic plot had the highest R^2 values. Thus, this kinetic model was taken as the best-fitted model for the description of the mechanism of color sorption of final discharge POME onto macrocomposites. In addition, examination of the sorption capacity values (q_e) of the pseudo-first-order model showed that the value was in the same range as the experimental sorption capacity values.

3.4. Treatment of final discharge POME

3.4.1. COD

COD is one of the main parameters to assess concentration of organic pollutants in wastewater. COD is used to measure the total quantity of oxygen-consuming substances in the complete chemical breakdown of organic substances in water. High COD level showed higher amount of carbon-containing compounds in the sample. From Fig. 6, the best COD reduction achieved was 93% (COD removal of 888 mg/L) using biofilm-coated macrocomposites. Slightly lower reduction of COD was observed using macrocomposites only (COD removal of 734 mg/L) without the biofilm.

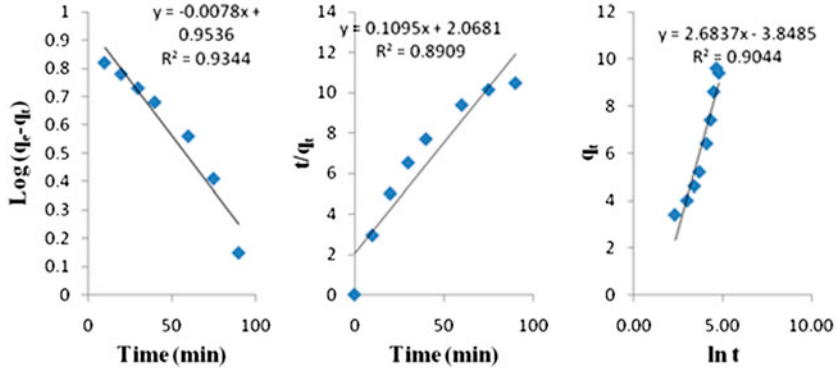

Fig. 5. *Left* Linear graph log (q_e−q_t) vs. t (Pseudo-first-order). *Middle* Linear graph $\frac{t}{q_t}$ vs. t (Pseudo-second-order). *Right* Linear graph q_t vs. ln t (Elovich).

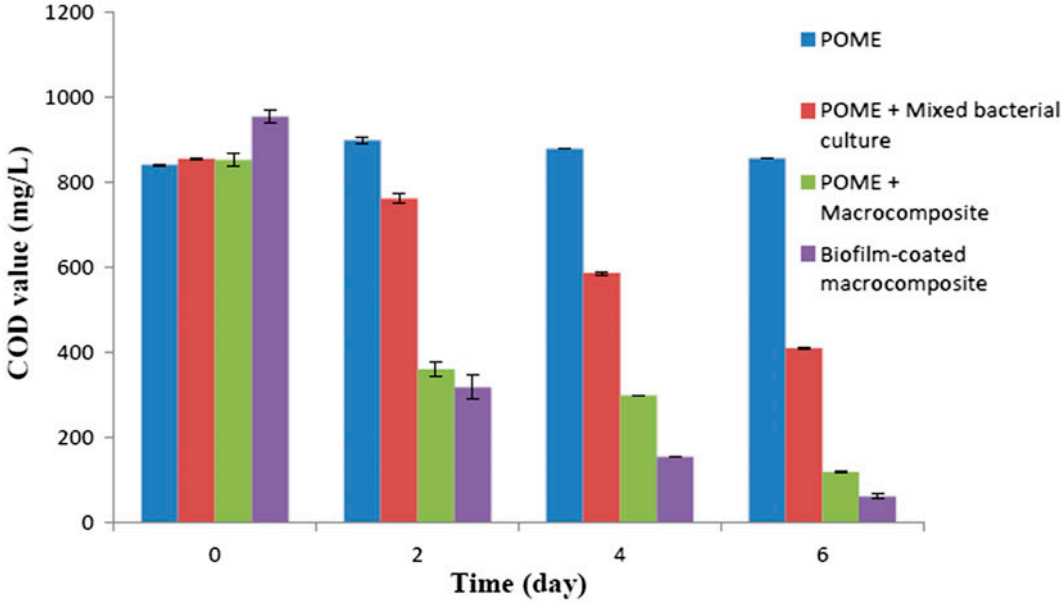

Fig. 6. COD profile with respect to time (day).

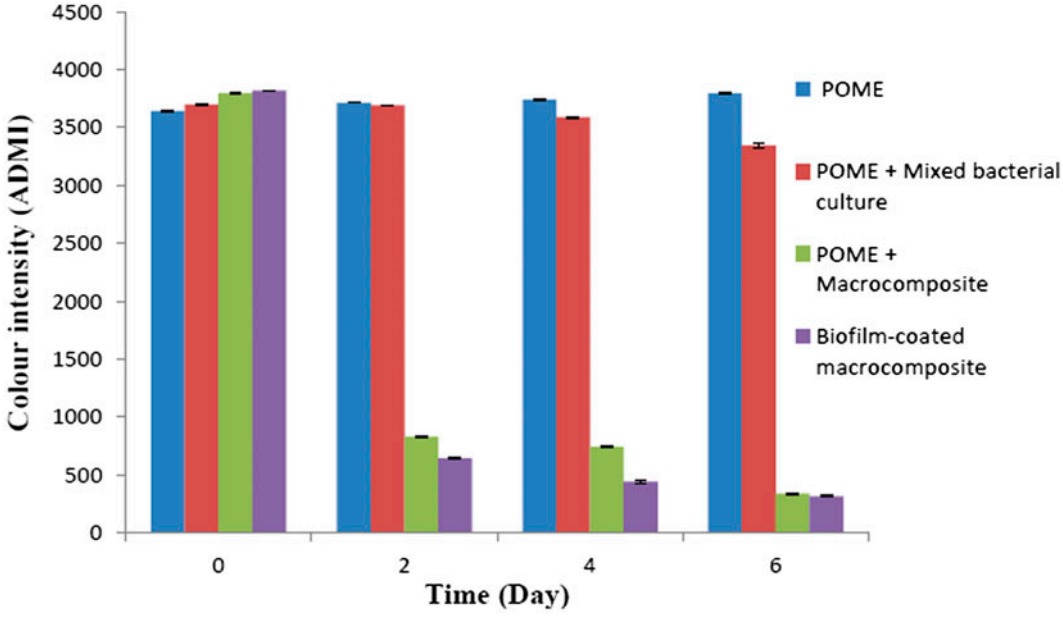

Fig. 7. Color profile with respect to time (day).

Comparatively, suspended cells of the mixed bacterial culture can only remove 52% (445 mg/L) of the COD. It was clearly seen that both the bacteria and the macrocomposite played important role in reducing COD of POME. The combination of bacteria and macrocomposite in a form of biofilm-coated macrocomposite improved the COD removal of POME. It can be implied that the mixed bacterial culture could utilize nutrients (carbon and nitrogen sources) found in POME for their growth which in turn reduce the COD. Biofilm-coated macrocomposites showed better results for the COD removal. The adsorption process by the macrocomposites and the ability of the immobilized mixed bacterial culture to metabolize organic compounds found in POME (rich source of nutrients such as carbohydrates, lipids, and proteins) contributed to high efficiency of COD reduction. Biofilm-forming microorganisms showed better

performance as compared to free-floating systems [14]. In particular, macrocomposites containing activated carbon is the most efficient method for COD removal because of the wide range of pore size distribution, high surface area, and hydrophobic properties to adsorb organic pollutants [15].

3.4.2. Color

In this study, biofilm-coated macrocomposites showed the highest color reduction of 92% (initial ADMI was 3514) compared to the use of only macrocomposites (91%). Meanwhile, POME treated with mixed bacterial culture in suspension showed a small reduction of color (10%). According to Fig. 7, the highest decolorization process was measured in the early phase until day 2 after which there was no significant change in color removal. This behavior can be explained by the availability of adsorption sites on the surface of macrocomposites. The adsorption sites were saturated by the adsorbate (POME component) and mixed bacterial culture that were immobilized on the macrocomposites. The lower efficiency of decolorization process could possibly be due to the fewer adsorption sites available [16]. The decrease in color was closely related to reduction of phenolic compounds and lignin. Polyphenols were found to contribute to color in POME. Polymerization of tannins and low molecular weight of phenolic compounds could also contribute to color of POME.

4. Conclusion

Biofilm-coated macrocomposite mixed culture consisting of *K. pneumonia* (MABZ) *E. faecalis*, *B. panacihumi*, and *L. fusiformis* demonstrated the ability to reduce color and COD. To further improve their performance, optimization color removal from POME is recommended for further study.

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