

Colour and COD Removal from Palm Oil Mill Effluent (POME) Using *Pseudomonas Aeruginosa* Strain NCIM 5223 in Microbial Fuel Cell

Hassan Sh Abdirahman Elmi*, Muhamad Hanif Md Nor and Zaharah Ibrahim

Environmental Biotechnology, Amoud University, Borama, Somalia

Abstract

Palm oil industries are the largest agricultural based industries in Malaysia and in processing palm oil, high pollutant liquid waste known as palm oil mill effluent (POME) is being generated. Currently, treatment of POME to meet the standard discharge has become an important issue. Therefore, this study was conducted to treat final discharge POME in microbial fuel cell (MFC). Double chamber MFC fabricated using polyacrylic sheets with a working volume of 1 L, proton exchange membrane (Nafion 115) were used. The anodic solution consisted of final discharge pond POME, overnight *Pseudomonas aeruginosa strain NCIM 5223* inoculum (10% v/v) and phosphate buffer (pH 7) while the cathodic solution consisted of phosphate buffer (pH 7) and potassium hexacyanoferrate (III). The results showed 58% of COD removal and 60% of colour removal in 8 days. In conclusion *Pseudomonas aeruginosa strain NCIM 5223* was able to remove colour and COD from final pond POME.

Keywords: Microbial fuel cell; COD; Wastewater treatment; POME; ADMI

Introduction

Palm oil tree (*Elaeis guineensis*) is an equatorial plant rich in edible oils belonging to the family of Palmae. In the last decade, cultivation of palm oil increased rapidly in Malaysia and as reported it covered more than 11% of the land in 2003 [1]. Palm oil tree is planted in nursery and transferred to the plantation where it reaches the first harvesting time of 3 years [2]. Currently, Malaysia and Indonesia are the largest palm oil producing countries in the worlds palm oil export [3]. Palm oil industries are the largest agro based industries in Malaysia and the production of crude palm oil increased from 10.6 million tonnes in 1999, to more than 17.7 million tonnes in 2008, then from more than 45.9 million tonnes in 2010, to more than 50.2 million in 2011 tonnes [4,5]. In extracting crude palm oil, more water is used and liquid waste known as Palm oil mill effluent (POME) is being generated [6,7]. Wet palm oil milling process is the extraction method of crude palm oil from the Fresh Fruit Bunches (FBB) adapted in Malaysia [8]. Wet milling process consists of several stages including sterilisation, stripping, digesting, and oil extraction generating huge quantities of POME wastewater. Raw POME has many characteristics including that it is acidic in pH, brownish in colour, and contain environmental pollutant elements such as; COD, BOD, total solids, suspended solids, oil and grease [3]. Discharging POME without proper treatments can cause problems to the environment [9]. For this reason, Malaysian government has set Environmental Quality Act 1917 which defines the standard discharge limit of effluent. Biological treatment is the common treatment method of POME adopted in Malaysia though other treatments such as; physicochemical and membrane filtration is considered. Improving treatment methods of POME can contribute to minimize environmental pollution. In this study a double chamber microbial fuel cell separated by proton exchange membrane was fabricated from polyacrylic sheets for the removal of chemical oxygen demand (COD) and colour from palm oil mill effluent (POME) in facultative anaerobic condition. POME and phosphate buffer was filled in the anode chamber while potassium hexacyanoferrate (III) and phosphate puffer was filled in the cathode chamber.

Methods and Materials

POME sampling and preparation

POME sample was collected from palm oil milling in Sedinak, Johor and stored at 4°C. Then POME was centrifuged at 4000 rpm for 15 minutes to remove the suspended solid. Then the supernatant was autoclaved at 121°C, 15 psi for 15 minutes to sterilize before it was used for treatment in the MFC. The process of sterilization was to kill indigenous microorganisms in the POME. Nine biological replicates were done during this study each with its control.

Preparation of the inoculum

Many colonies were isolated form POME sludge using bacterial isolating techniques. *Pseudomonas aeruginosa strain NCIM 5223* was among the best once in treating POME during the pre-testing process.

Designing MFC and analysis

As Figure 1 shows double chamber MFC was fabricated using polyacrylic sheets with a working volume of 1 L, proton exchange membrane (Nafion 115) were used. The anodic solution consisted of autoclaved final discharge pond POME, overnight *Pseudomonas aeruginosa strain NCIM 5223* inoculum (10% v/v) and phosphate buffer (pH 7) while the cathodic solution consisted of phosphate buffer (pH 7) and potassium hexacyanoferrate (III). After that, 10 mL of POME was removed in time interval from the MFC to analyze the removal of colour and COD from POME using APHA method on HACH DR 5000 [10].

***Corresponding author:** Hassan Sh Abdirahman Elmi, Environmental Biotechnology, Amoud University, Borama, Somalia, Tel: +252634457106; E-mail: rabiic23@hotmail.com

Received February 02, 2015; **Accepted** August 14, 2015; **Published** August 14, 2015

Citation: Elmi HSA, Nor MHM, Ibrahim Z (2015) Colour and COD Removal from Palm Oil Mill Effluent (POME) Using *Pseudomonas Aeruginosa* Strain NCIM 5223 in Microbial Fuel Cell. J Waste Resources 5: 181. doi: [10.4172/2252-5211.1000181](https://doi.org/10.4172/2252-5211.1000181)

Copyright: © 2015 Elmi HSA, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

DNA extraction

Isolation of genomic DNA was carried out according to Promega extraction kit (Wizard® Genomic DNA Purification Kit). Extracted DNA and 1 kb DNA ladder was loaded into 1% (w/v) polyacrylamide gel prepared from 0.50 g of agarose powder dissolved into 50 mL of 1 x TAE buffer stained with Ethidium Bromide (EtBr). Electrophoresis was run in 1 x TAE buffer at 90 V for 60 minutes.

Polymerase Chain Reaction (PCR)

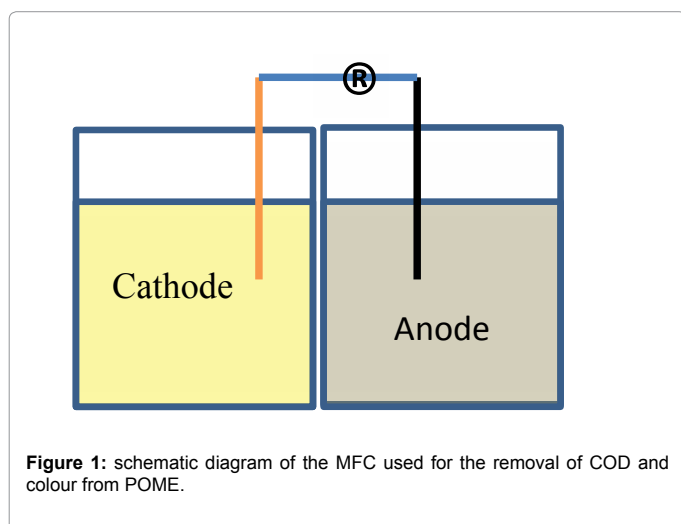
Bacterial 16S rRNA gen was amplified by PCR using primers Fd1 (5'-AGA GTT TGA TCC TGGCTC AG-3') with 50% CG clamp and rp1 (5'-ACG GCT ACC TTG TTA CGA CTT-3') the PCR reaction and the thermal cycles were carried out as described by [11]. PCR products were again loaded in 1% (w/v) polyacrylamide gel then sent for sequences. Obtained sequences were compared with NCBI genomic database through BLAST and the phylogenetic tree were constructed using MEGA version 5 [12].

Result and Discussion

COD removal

The chemical oxygen demand (COD) indicates organic pollutants in the wastewater. Bacteria oxidises organic compounds in the wastewater for their growth and metabolism. The initial concentration of COD in the final discharge pond was 991 mg/L before the treatment and the final concentration of COD after the treatment was 441 mg/L. So *Pseudomonas aeruginosa* strain NCIM 5223 was able to remove 550 mg/L of the COD (58%) in 8 days. Figure 2 shows, the percentage of COD removal in the MFC. According to [13] development of biofilm increase COD removal performances compared to the suspended system. *P. aeruginosa* strain NCIM 5223 was developed biofilm on the anode and as the bacterial growth increased removal of the COD also increased. Significant COD removal was observed in the first three days of the treatment comparing to other days of the treatment. Similarly *P. aeruginosa* strain NCIM 5223 reached the maximum growth in the first 3 days of the experiment. From the glucose concentration result more than 90 % of the glucose content was used up in the first 3 days. Therefore the relationship between the COD removal, *P. aeruginosa* strain NCIM 5223 growth and the glucose removal was directly related.

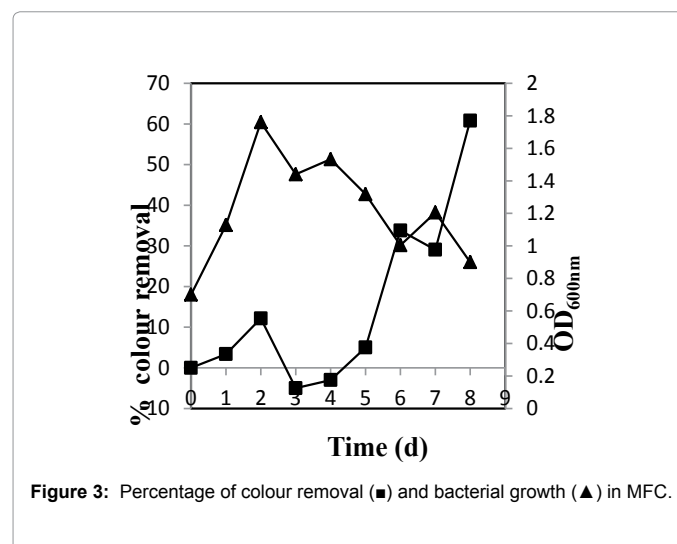
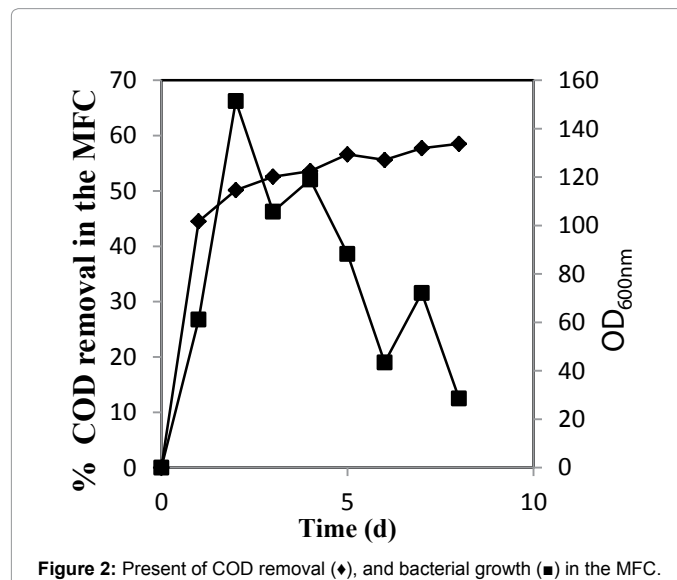
Colour removal



The American dye manufacture intensity (ADMI) is the method of measuring colour intensity in the wastewater. The initial colour concentration in the final pond POME was 4440 ADMI and the final colour concentration of POME was 1740 which means 2700 ADMI (60 %) of the colour was removed. As show in Figure 3, there was no significant ADMI removal in the first days of the treatment. However; after the 4th day of the treatment the removal of ADMI was very high. This indicates that when almost all the glucose in the system was used bacteria started to remove the colour of the effluent. From the literature colour of the effluent is mainly contributed by the organic compounds, using these organic compounds can remove the colour of the effluent [14].

Bacterial analysis

Bacterium isolated was gram positive having morphological appearances of coccus when stained. Then genomic DNA was extracted following Promega extraction kit and run the PCR. After that the PCR product was sequenced and the obtained results were BLAST and found that the bacterium was *Pseudomonas aeruginosa* strain NCIM



5223. Figure 4, shows the phylogenetic tree of 15 different *Pseudomonas* groups. During the experiment it was observed that the bacterium was developed biofilm on the electrode and as reported by [3] biofilm formation improves the COD removal from the effluent [7,9,12].

Conclusion

Treatment of the final discharge POME was successfully conducted focusing on colour and COD removal using APHA method. The maximum COD removal achieved was 58% from the initial reading of 991 mg/L while the colour reduction was 60% from the initial reading of 4440 ADMI in 8 days.

References

1. Ahmad AL, Ismail S, Ibrahim N, Bhatia S (2003) Removal of suspended solids and residual oil from palm oil mill effluent. *Journal of Chemical Technology and Biotechnology* 78: 971-978.
2. APHA, AWWA, WEF (2005) Standard methods for the examination of water and wastewater. (21st ed.) Washington D.C: American Public Health Association.
3. Assas N, Ayed L, Marouani L, Hamdi M (2002) Decolorization of fresh and stored-black olive mill wastewaters by *Geotrichum candidum*. *Process Biochemistry* 38: 361-365.
4. Cappuccino J, Sherman N (2002) *Microbiol. a laboratory manual*. Pearson Education, Inc. San Francisco, CA.
5. Ibrahim AH, Dahlan I, Adlan MN, Dasti AF (2012) Comparative Study on Characterization of Malaysian Palm Oil Mill Effluent *Research Journal of Chemical Sciences* 2: 1-5.
6. Jia C, Xiuping Z, Jinren N, Alistair B (2010) Palm oil mill effluent treatment using a two-stage microbial fuel cells system integrated with immobilized biological aerated filters. *Bioresource technology* 101: 2729- 2734.
7. Li XN, Song HL, Li W, Lu XW, Nishimura O (2010) An integrated ecological floating-bed employing plant, freshwater clam and biofilm carrier for purification of eutrophic water. *Ecological Engineering* 36: 382-390.
8. Ma A (2000) Environmental management for the palm oil industry. *Palm Oil Dev* 30: 1-10.
9. Neoh CH, Lam CY, Lim CK, Yahya A, Ibrahim Z (2013) Decolorization of palm oil mill effluent using growing cultures of *Curvularia clavata*. *Environmental Science and Pollution Research* 21: 4397- 4408.
10. Neoh CH, Yahya A, Adnan R, Majid ZA, Ibrahim Z (2012) Optimization of decolorization of palm oil mill effluent (POME) by growing cultures of *Aspergillus fumigatus* using response surface methodology. *Environmental Science and Pollution Research* 20: 2912- 2923.
11. Rupani P, Singh R, Ibrahim M (2010) Review of current palm oil mill effluent (POME) treatment methods: Vermicomposting as a sustainable practice. *World Applied Sciences* 11: 70:81.
12. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, et al. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular biology and evolution* 28: 2731-2739.
13. Wood BJ, Pillai KR, Rajaratnam JA (1979) Palm oil mill effluent disposal on land. *Agricultural Wastes* 1: 103-127.
14. Wu TY, Mohammad AW, Jahim JM, Anuar N (2010) Pollution control technologies for the treatment of palm oil mill effluent (POME) through end-of-pipe processes. *Journal of Environmental Management* 91: 1467-1490.