DECOLORIZATION OF PALM OIL MILL EFFLUENT USING SELECTED INDIGENOUS BACTERIA

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To Allah S.W.T

…Your Mercy has been always surpasses all understanding…

To my parents and family

…Your hopes and wishes has been the greatest poem in my life…

To my lecturer

…Your guardian has made all these become possible…

To my friends

…Thanks a lot…

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ABSTRACT

In this study, the selection of suitable bacteria that is best to treat POME from final pond is the main objective. There are 20 bacteria that had been isolated from final pond of POME but only 11 species has the ability to reduce color of POME. However, only five strains out of 11 species were capable to reduce the color after 6 days of incubation compared to others which took longer time. Bacteria D showed the best results in reducing the color of POME which was 45% (from 4437 to 2448 ADMI) after 6 days of treatment. Partial identification of bacteria D using biochemical test showed that it belonged to *Proteus inconstans*. Results showed that POME wastewater treated with indigenous bacteria D successfully reduced the COD level up to 61 % (from 34170 to 13464 mg/L), pH value from 9.25 to 9.11 at day 2, 19% of lignin contents (from 310 to 250 mg/L), 17% of phenolics compound (from 271 to 225 mg/L), 47% (from 5202 to 2754 ADMI) of color intensity (ADMI) respectively. The highest lignin peroxidase activity was 1.94 U/mL implying the optimum bacterial activity occurred during the exponential growth phase. The study suggested that isolated indigenous bacteria could decolorize POME wastewater.

ABSTRAK

Dalam kajian ini, pemilihan bakteria yang sesuai yang terbaik untuk merawat POME dari kolam akhir adalah objektif utama. Terdapat 20 bakteria yang telah diasingkan daripada kolam akhir POME tetapi hanya 11 spesis yang berupaya untuk mengurangkan warna POME. Walau bagaimanapun, hanya lima jenis daripada 11 spesis mampu mengurangkan warna selepas 6 hari perawatan berbanding dengan lain-lain yang mengambil masa yang panjang. Bakteria D menunjukkan hasil yang terbaik dalam mengurangkan warna POME yang merupakan 45% (4437-2448 ADMI) selepas 6 hari rawatan. Separa pengenalan bakteria D menggunakan ujian biokimia menunjukkan bahawa ia kepunyaan *Proteus inconstans*. Keputusan menunjukkan bahawa air sisa POME dirawat dengan bakteria D berjaya mengurangkan tahap COD sehingga kepada 61% (dari 34170 ke 13464 mg / L), nilai pH dari 9.25 ke 9.11 pada hari ke2, 19% kandungan lignin (dari 310 ke 250 mg / L), 17% komponen fenol (dari 271 ke 225 mg / L), 47% (dari 5202 ke 2754 ADMI) kepekatan warna (ADMI). Lignin peroxidas aktiviti yang paling tinggi adalah 1.94 U / mL yang memantulkan peringkat optimum bakteria aktiviti yang merupakan fasa eksponen pertumbuhan bakteria. Hasil kajian mencadangkan bahawa bakteria asli boleh mengurangkan warna air sisa POME.

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LIST OF ABBREVIATIONS

ADMI	American Dye Manufacturers Institute
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
NB	Nutrient Broth
WQI	Water Quality Analysis Index
TSI	Triple Sugar Iron
MR-VP	Methyl Red- Voges Proskauer
O-F	Oxidation- Fermentation
H_2O_2	Hydrogen Peroxide
$K_2Cr_2O_7$	Potassium dichromate
$CaCI_2$	Calcium chloride
$MgSO_4$	Magnesium sulphate
KH_2PO_4	Potassium dihydrogen phosphate
NaOH	Sodium hydroxide
$(NH_4)_3SO_4$	Ammonium sulphate
$AgSO_4$	Silver sulphate
$HgSO_4$	Mercury sulphate
H_2SO_4	Sulphuric acid

LIST OF SYMBOLS

L	Liter
mL	Milliliter
g/L	Gram per liter
et al.	And friends
sp.	Species
v/v	volume over volume

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CHAPTER 1

INTRODUCTION

1.1 Research background

Palm oil is one of the most important vegetable oil in the world (Hartley, 1988). Neoh *et al* (2012) has reported that the second world largest palm oil producer after Indonesia was Malaysia; with the amount of 10.6 million tonnes of palm oil production as far back as in year 1999 and this amount has becomes increasing to 17.7 million tonnes in year 2008 and expected to rise every year. The increasing of the palm oil production generally contributed to increase the different kind of waste such as solid waste; in example is empty fruit bunch and liquid waste like POME (Igwe and Onyegbado, 2007).

Palm Oil Mill Effluent also known as POME is an effluent that is large in quantity resulting from the process of extracting the crude palm oil from the fresh fruit bunch (Ahmad *et al*., 2003). It consists of colloidal suspension of 95-96% water, 0.6-0.7% oil, and yet, 4-5% total solids including 2-4% suspended solids originating from the mixture of a sterilizer condensate, separator sludge and hydrocyclone wastewater (Ma, 2000; Ahmad *et al*., 2003)

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Studies have reported that POME also consists of soluble materials such as CH_4, SO_2, NH_3, halogens or soluble liquids or solids which contain ions of either organic or inorganic with their concentration were over the standard limit of threshold value (Igwe and Onyegbado, 2007). Consequently, the discharged of POME directly to nature will harm aquatic life, also the environment.

Even though it is not highly polluted, the wastewater is harmful if directly released to the environment due to its high chemical oxygen demand (COD), biochemical oxygen demand (BOD), phenols and color concentration. as well as unpleasant odors (Poh *et al*., 2009). There is no chemical addition during palm oil mill processing. However, the appeared color during discharged of POME was derived from plant constituents such as lignin and phenolic compounds.

Recent study showed that phenolics (tannic and humic acids) from the feedstock and melanoidins from Maillard reaction of sugars (carbohydrates) with proteins (amino groups) might contribute to the color of the sugar processing effluent. Consequently, this sugar processing effluent was then mixed together with other wastes and discharged through POME (Zahrim *et al*., 2009).

The discharge of color effluents directly to water may affect aquatic life as it will reduce the penetration of sunlight that will inhibit the photosynthetic activity inside water (Mohan and Karthikeyan, 1997; Neoh *et al.*, 2012). Yet, the colored compounds from POME may chelate with metals ions inside water and result in toxicity to aquatic organisms as reported by Mohan and Karthikeyan (1997) and Neoh *et al* (2012). Indeed, the presence of humic compounds will react with chlorine may produce carcinogenic byproducts like trihalomethanes (Vukovic *et al*., 2008; Neoh *et al*., 2012). Other than that, studies have reported that the compounds that were derived from lignin might inhibit the development of embryo of aquatic organisms (Pillai *et al*., 1997; Neoh *et al*., 2012).

Since conducting an experiment based on water contamination with palm oil mill effluent (POME) has recently attracted much attention, decolorization of palm oil mill effluent using selected indigenous bacteria had been carried out in this study. The dominant bacteria for POME color removal were more focused and partially identified after Gram staining and biochemical test were performed and these bacteria were then applied to POME waste to assess their effectiveness in treating POME wastewater.

1.2 Problem statement

As mentioned earlier, huge quantities of waste such as empty fruit bunch and POME are produced in the palm oil mill industry. There was chemical and physical methods that were applied to treat POME wastewater included adsorption, coagulation-flocculation, oxidation and electrochemical method. However, these methods are highly expensive, formation of hazardous byproducts or intensive energy requirements (Couto, 2009; Chanida and Poonsuk, 2011).

Another alternative way is by using biological treatment. The biological method is more effective in treatment and it sufficiently needs to apply due to cost-effective and environmental friendly (Zahrim *et al*., 2009).

Previous study had shown that the use of white-rot fungi were capable to decolorize and phenol removal from wastewaters (Kissi *et al*., 2001). But, Akin and Benner have reported that the anaerobe rumen bacteria could solubilize lignin better than the rumen fungi (Hiroshi *et al*., 2000). In addition, there is biological treatment that utilized aerobic and anaerobic; results showed that the aerobic treatment was more efficient compared to anaerobic (Anggelis *et al*., 2001). Due to ability to degrade organic material as reported by Epstein (1997) and Ipek *et al* (2002), aerobic microorganisms were applied in this study.

1.3 Objectives of the research

The objectives of this study were:

i. To isolate and partial characterize the selected indigenous bacteria present in palm oil mill effluent that had roles in POME treatment.

ii. To investigate the effectiveness of isolated microorganisms in treating POME such as chemical oxygen demand (COD), pH, total phenolic compounds and determining lignin content.

iii. To examine POME color removal (ADMI unit) after selected bacteria were applied

1.4 Research significance

The importance of this study was to isolate and characterize the selected bacteria from POME that had roles in POME treatment. This is very important to isolate indigenous microorganism with specific enzyme that can be used for the treatment. The effectiveness of isolated microorganisms in treating POME such as chemical oxygen demand (COD), pH, total phenolic compounds and lignin were determined. These tests were used to compare between treated and untreated sample after the selected bacteria were applied. This is very important to analyze the effectiveness of isolated bacteria in treating POME. The study was then followed by analyzing the color reduction of POME in order to solve unwanted color present by using effective microorganisms.

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