Bio-remediation of palm oil mill effluent (POME) using *Aspergillus niger* immobilized on coconut husk and free cell fermentation

P Thegarathah¹, J Jewaratnam¹ and K Simaranı²

¹ Department of Chemical Engineering, Faculty of Engineering, University of Malaya
² Institute of Biological Science, Faculty of Science, University of Malaya

Abstract. *Aspergillus niger* which is ubiquitous and acts as good biological agent has been chosen to treat POME in this study. Use of, free cell fermentation limits the uptake of substrate as compared to immobilization method which provides larger surface area for cell growth. Therefore, the idea of using coconut husk as immobilization medium has been proposed. Coconut is widely available in Malaysia almost at no cost. Hence, the purpose of this study is to evaluate the bio-degradation of palm oil mill effluent by *Aspergillus niger* through immobilization on coconut husk and free cell fermentation. Substrates from different types of POME such as, raw, sterile, treated using *Aspergillus niger* through free cell fermentation and treated with *Aspergillus niger* cells which are immobilized on coconut husk were prepared at different concentration range from 0 to 100%. *Aspergillus niger* cells (1 X 10⁵ cells per ml were inoculated into Erlenmeyer flasks and incubated at 30 ± 2 °C and 150 rpm for seven days. Fermentation slurry were used to analyse for turbidity, COD and pH. The results were compared with free cell fermentation method. Raw POME has significantly low turbidity as compared to sterile and treated POME. Autoclaving POME increases the turbidity. 100% sterile POME shows the highest turbidity which is 979.5 FAU and 100% POME which was treated by immobilization on coconut husk shows a reduction in turbidity which gives 900.5 FAU. The presence of coconut husk has significantly increased the COD value as 0% POME (distilled water) with coconut husk shows 3320 mg/l of COD. However, at the end of seven days of treatment, 100% POME which was treated through immobilization shows lower COD as compared to free cell fermentation. pH reading is a concern as treatment method might influence the pH of POME. Range of pH for POME throughout the experiment was between 7.1 and 9.6. Degradation of POME using *Aspergillus niger* immobilized on coconut husk provides additional incentive that is cheap and sustainable.

Key Words: Bio-remediation, Fermentation, Palm Oil Mill Effluent, Industrial Waste Water, Fungal Biotechnology, Biological Treatment, *Aspergillus niger*

1. Introduction

Palm oil is a promising source of cooking oil which has been at high demand [1] since the 14th century [2]. Malaysia is one of the largest palm oil producers worldwide. Palm oil has been contributing to the socio-economic growth of a country. Palm oil processing in Malaysia produced 30 million tonnes of POME in the year of 2004 [3]. Environmental issues are alarming over the recent years. Oil industry has been responsible for the release of waste effluent which results in huge impact on the ecology [4]. POME is one of the effluents which has vast harmful impact on the environment [1] as land and aquatic pollutant. POME is a brownish liquid with high amount of total suspended solid (TSS), oil and grease, chemical oxygen demand (COD), and biological oxygen demand (BOD). For instance, POME alters soil’s physicochemical characteristics. This is due to its high organic content and some organic acids [4]. Existing treatment which is practiced by most of the mills are ponding or open digesting tank system. Unfortunately, these treatment methods take up huge space, long retention time, and yield in odororous and corrosive gases [5]. Added to these drawbacks, existing methods are also not meeting the standard of discharge limits set by Department of Environment [6] as stated in Table 1 [7].
Table 1. Acceptable Conditions for Discharge of Industrial Effluent for Mixed Effluent

| Parameter          | Unit | Standard A | Standard B |
|--------------------|------|------------|------------|
| Temperature        | ℃    | 40         | 40         |
| pH value           |      | 6.0-9.0    | 5.5-9.0    |
| BOD at 20℃        | mg/l | 20         | 40         |
| Suspended solids   | mg/l | 50         | 100        |
| Oil and grease     | mg/l | 1.0        | 10         |
| Ammoniacal nitrogen| mg/l | 10         | 20         |
| Colour             | Pt-Co| 100        | 200        |

Besides, other treatment methods like integrated aerobic-anaerobic bio-reactor, up-flow anaerobic sludge bio-reactor and anaerobic bench scale reactor require high implementation cost [6]. That is the main reason for biodegradation to be a high demand venture in this current era. Biodegradation is a biological treatment through attached or suspended growth of microorganisms [8] like Trichoderma viride, [9] and Aspergillus niger [10]. Aspergillus niger which is known as black Aspergilli is a filamentous fungus which can degrade and transform the toxic organic content into disposal waste [6]. Black Aspergilli is remarkably found in tropical and sub-tropical regions which grows in organic substrate or medium at aerobic condition as they cause date rot and onion rot. Aspergillus acts as a good decomposer [11]. It has also been widely applied in food industry as they are tagged as GRAS (Generally Regarded as Safe). A cost effective and environmentally friendly treatment method is certainly in need. The main reason of this study is to provide the fundamental data of POME treatment by Aspergillus niger. The performance and treatability of Palm Oil Mill Effluent (POME) was examined using Aspergillus niger from two different approaches. Free-cell fermentation was compared with immobilization of Aspergillus niger cells using coconut husk as another low cost agro-industrial by-product. The usage of coconut husk is the novelty of this study. Hence, this cost-effective and sustainable approach has been investigated.

2. Materials and Method

2.1. Sample collection and microorganism

Raw palm oil mill effluent (POME) was collected from Sime Darby Research Sdn. Bhd, Carey Island Selangor, Malaysia at temperature ranging from 80 to 90 ℃. It was then stored in clean containers under room temperature. Aspergillus niger which was obtained from the stock culture of Microbiology Department, Institute of Biological Science, Faculty of Science at University of Malaya was used in this study. The fungal strain was reactivated by sub-culturing on the fresh potato dextrose agar (PDA) plate and incubated at 28 ± 2 ℃ for 7 days. Spores were then scraped using sterilized spatula and resuspended in the sterilized distilled. Spore suspension stock was prepared to 1x10⁵ cells per ml and kept at 4 ℃ for further use.

2.2. Free-Cell Fermentation

Bio-remediation experiments were carried out in 200 ml conical flasks containing 100 ml of fermentation media (POME) at different concentration ranging from 0-100%. 1 ml of Aspergillus niger spore suspension was inoculated into each flask followed by incubation on rotary shaker at 150 rpm at 30 ± 2 ℃ for 7 days. Each experiment set was run in triplicates. The fermentation slurry was harvested and used to carry out post-treatment analysis of POME.

2.3. Immobilized-Cell Fermentation

The pieces of coconut husk were cleaned and air dried. They were then further dried in an oven at 105 ℃ for 2-3 hours till the mass obtained becomes constant. The dried pieces were then autoclaved at 121 ℃ (15 psi) for 20 minutes before being introduced into 200 ml conical flask containing 100 ml of sterile POME at different concentration. After cooling, each medium containing flask was inoculated.
with *Aspergillus niger* spore suspension. This was followed by incubation on rotary shaker at 150 rpm at 30 ± 2 °C for 7 days. The supernatant was used to carry out post-treatment analysis of POME.

### 2.4. Post treatment Analyses

Analyses were carried out on raw, sterile, and treated POME samples. Chemical Oxygen Demand (COD) analysis was done using a portable calorimeter (DR/850) by Hach USA. Pre and post treatment analysis were done according to method 8000 for water, wastewater, and seawater provided by Hach with some alteration using COD digestion reagent vials (high range plus). Turbidity (FAU) analysis was also done using a portable calorimeter (DR/850). It was done according to method 8237 for water, wastewater, and seawater provided. pH analysis was done using a pH meter.

### 3. Results and Discussion

The performance and treatability of Palm Oil Mill Effluent (POME) by using *Aspergillus niger* using two different approaches i.e. fermentation using free-cell and immobilization using coconut husk were investigated. The three parameters which were analysed are COD, turbidity and pH of treated POME.

#### 3.1. COD Analysis

Raw POME sample was tested for its COD reading at different concentrations of 0%, 20%, 40%, 60%, 80% and 100%. 20% of POME gave an average reading of 4690 mg/l of COD and 100% of POME contained 16500 mg/l of COD. The same analysis was done on the POME from the same source of sample once it was made sterile through autoclaving at 120°C (15psi) for 20 minutes.

![Figure 1: Profile of COD (mg/l) versus POME concentration at different conditions](image_url)

Sterilisation of POME sample significantly decrease the COD of the sample. 20% of sterile POME shows 3510 mg/l of COD which shows 25% decrease in COD as compared to raw POME sample. This proves that sterilising biological waste decreases it chemical oxygen demand. Therefore, sterile POME was used to compare the treatment method which involved free-cell fermentation and immobilized cell fermentation. Immobilization in this study was done using sterile coconut husk. Using sterile coconut husk has a huge increase in COD as compared to free cell fermentation where they contain 3310 mg/l and 10 mg/l of COD respectively. On the other hand, 100% POME which was treated with coconut husk shows lower COD, but they differ by only 130 mg/l. COD of the treated POME could be higher due to the consumption of glucose in POME [12].
3.2. Turbidity Analysis

Turbidity of POME increases with concentration and plateau at different points for each condition. In overall, sterile POME has the highest turbidity as it was placed under high temperature and pressure during autoclaving.

![Graph showing turbidity (FAU) versus POME concentration at different conditions]

Figure 2: Profile of turbidity (FAU) versus POME concentration at different conditions

High pressure causes particles to break and move apart which increases the turbidity of the medium. Both fermentation methods reduce the turbidity of sterile POME. Immobilization method was able to reduce turbidity by 8.1% while free cell fermentation method has reduced the turbidity by 7.3%. Higher agitation rate increases turbidity. 150 rpm is considered high and predicted to increase the turbidity of POME [6]. This can be due to the trapped solid particles which apart at higher agitation rate [13].

3.3. pH analysis

The impact of fermentation on pH was analysed. Concentration of POME has no significant impact on pH. The range was between 8.2 to 9.7 for 20% to 100% of POME which indicates that the medium was alkaline.

![Graph showing pH versus POME concentration at different conditions]

Figure 3: Profile of pH versus POME concentration at different conditions

Sterilising POME sample increases its pH. The plot shows that raw POME has lower pH as compared to sterile POME. This pH was reduced by treatment using A. niger. Comparatively,
POME treated using immobilization shows significant reduction in pH. This can be due to production of acidic metabolites like citric acid, oxalic acid, and gluconic acid by *A. niger*. Consumption of glucose will also result in slight pH drop [12].

4. Conclusion
The results obtained in this study indicated the presence of coconut husk increases COD, yet immobilized cell treatment method reduces COD in 100% POME by 4%. Sterilisation process increases turbidity and pH and immobilization technique reduces it. Therefore, immobilized *Aspergillus niger* cells fermentation method using coconut husk can be used as an additional incentive that is cheap and sustainable.

5. Acknowledgement
The authors gratefully express their sincere thanks to the Department of Chemical Engineering, Faculty of Engineering and Institute of Biological Science, University of Malaya for their kind support.

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