BIODEGRADATION OF FRESH PALM OIL MILL EFFLUENT AND FUNGAL BIOMASS PROTEIN RECOVERY BY *ASPERGILLUS NIGER* AND *TRICHODERMA VIRENS*

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To my beloved father and mother;

Jalaludin bin Hj Md Yusof and *Sadaah Muhammad*

Brother and sisters;

For their love, support and best wishes

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ABSTRACT

This study was conducted to evaluate the potential of filamentous fungi for fresh palm oil mill effluent (POME) biodegradation. Two strains of filamentous fungi namely *Aspergillus niger* and *Trichoderma virens* were utilized and their performance in fresh POME biodegradation were studied and compared. The results of preliminary study showed that *A. niger* demonstrated high capacity in enhancing the biodegradation process of fresh POME in terms of reduction of total suspended solids (TSS) (340 mg/l), turbidity (110 NTU) and chemical oxygen demand (COD) (3200 mg/l) than *T. virens* (TSS: 720 mg/l; turbidity: 224 NTU; COD: 3520 mg/l). Additionally, considerable amount of biosolids enriched with fungal biomass corresponding to higher protein yield was achieved in fungal treated fresh POME as greater amount was obtained in fresh POME treated by *A. niger* (13.9 g/l/3.09 g/l) as compared to *T. virens* (9.95 g/l/2.33 g/l). Effects of inoculum size (%), agitation rate (rpm) and temperature (^{o}C) on the biodegradation process of the fresh POME by both fungal strains were examined using response surface methodology (RSM). Fresh POME treated by *A. niger* was more predictable compared to *T. virens* as high reduction of TSS and specific resistance to filtration (SRF) with greater recovery of biosolids enriched with fungal biomass (50.40 g/l) and protein (12.9 g/l) were achieved at optimal operating conditions. Monod kinetic model was utilized to define the biodegradation kinetic of fresh POME by both strains. High maximum specific growth rate (μ_{max}) (0.81 h $^{-1}$) revealed in fresh POME treated by *A. niger* thus signify that *A. niger* has the fastest growth rate and thus would be most effective in reducing the pollutant levels of fresh POME compared to *T. virens* (0.009 h^{-1}).

ABSTRAK

Kajian ini dijalankan untuk menilai potensi kulat filamen untuk biodegradasi efluen kilang kelapa sawit (POME) segar. Dua jenis kulat filamen iaitu *Aspergillus niger* dan *Trichoderma virens* telah digunakan dan prestasi mereka dalam biodegradasi POME segar telah dikaji dan dibandingkan. Hasil kajian awal menunjukkan bahawa *A. niger* menunjukkan kapasiti yang tinggi dalam meningkatkan proses biodegradasi POME segar dari segi pengurangan jumlah pepejal terampai (TSS) (340 mg/l), kekeruhan (110 NTU) dan permintaan oksigen kimia (COD) (3200 mg/l) berbanding kadar penyingkiran daripada *T. virens* (TSS: 720 mg/l; kekeruhan: 224 NTU; COD: 3520 mg/l). Selain itu, sejumlah besar biopepejal yang kaya dengan biomas kulat sepadan dengan hasil protein yang lebih tinggi telah dicapai dalam POME segar dirawat dengan kulat dengan jumlah yang lebih besar telah diperolehi dalam POME segar dirawat oleh *A. niger* (13.9 g/l/3.09 g/l) berbanding *T. virens* (9.95 g/l/2.33 g/l). Kesan saiz inokulum (%), kadar campuran (rpm) dan suhu (^{o}C) pada proses biodegradasi POME segar menggunakan kedua-dua jenis kulat telah diperiksa menggunakan kaedah permukaan sambutan (RSM). POME segar dirawat oleh *A. niger* adalah lebih mudah diramalkan berbanding *T. virens* dengan pengurangan TSS dan rintangan khusus untuk penapisan (SRF) adalah lebih tinggi dan juga penghasilan biopepejal yang kaya dengan biomas kulat (50.40 g/l) dan protein (12.9 g/l) telah dicapai pada keadaan yang optima. Model kinetik Monod telah digunakan untuk menentukan kinetik biodegradasi daripada POME segar oleh kedua-dua jenis kulat. Kadar pertumbuhan khusus maksimum (μ_{max}) (0.81 h^{-1}) yang tinggi yang ditunjukkan oleh POME segar dirawat oleh *A. niger* menunjukkan bahawa *A. niger* mempunyai kadar pertumbuhan paling pesat dan oleh itu berkesan dalam mengurangkan tahap pencemar akibat daripada POME jika dibandingkan dengan *T. virens* (0.009 h^{-1}).

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LIST OF ABBREVIATIONS

BOD	-	Biochemical Oxygen Demand
BSA	-	Bovine serum albumin
CCD	-	Central composite design
COD	-	Chemical Oxygen Demand
CPO	-	Crude Palm Oil
$CuSO_4.5H_2O$	-	Copper (II) sulphate pentahydrate
DOE	-	Department of Environmental
EFB	-	Empty Fruit Bunch
FBP	-	Fungal Biomass Protein
FFB	-	Fresh Fruit Bunch
$NaCO_3$	-	Sodium carbonate
NaOH	-	Sodium hydroxide
POME	-	Palm Oil Mill Effluent
POMS	-	Palm Oil Mill Sludges
PPNJ	-	Persatuan Perladangan Negeri Johor
RSM	-	Response surface methodology
SRF	-	Specific Resistance to Filtration
TSS	-	Total Suspended Solid
VSS	-	Volatile Suspended Solid

LIST OF SYMBOLS

^{O}C	-	Degree Celcius
µm	-	micrometer
g	-	gram
L	-	Liter
m^3	-	Cubic meter
mg	-	miligram
mL	-	mililitre
v/v	-	volume per volume
w/w	-	weight per weight
μ	-	growth rate constant
S	-	substrate concentration
Ks	-	substrate saturation constant
μ_{max}	-	maximum growth rate
X	-	biomass
R^2	-	determination of coefficient
β_i	-	linear coeffcient
β_{ii}	-	quadratic coefficient
β_{ij}	-	interaction coefficient
$Y_{x/s}$	-	yield coeffcient

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CHAPTER 1

INTRODUCTION

1.1 Research background

The oil palm fruit processing not only produce crude palm oil as a major product but also generates large quantities of liquid wastes which results in unmanageable environmental pollution. Moreover, the industrial processes can also lead to negative environmental impacts which can considerably cause climate change, loss of natural resources, air and water pollution and extinction of species. Besides, palm oil mill effluent (POME) is the highest single polluter of Malaysian rivers. An estimated of 30 million tons of POME are produced annually from more than 300 palm oil mills in Malaysia. POME discharge and the problems associated with it are already alarming and dangerously keep growing. Pollution problems caused by POME relate mainly to its oxygen depleting effects. It was found that the oxygen depleting potential of POME is 100 times greater than that of domestic sewage (Singh *et al*., 2010).

Currently, the most common method adopted by most palm oil processing-mills is biological treatment also identified as ponding system. The ponding system requires large area of land for their series of ponds so that the desired characteristics for the discharge are achieved (Hassan *et al*., 2004). In addition, vast amount of corrosive and odorous biogas containing methane, carbon dioxide, and a very small amount of hydrogen sulphide are generated which can adversely affect the environment (Rupani *et al*., 2010). Moreover, this commonly used conventional method is unable to meet the guidelines set by the Department of Environmental (DOE) especially in terms of COD and TSS concentration.

Previous researches had found that filamentous fungi have been utilized to treat wastewater ingredients such as metals, inorganic nutrients and organic compounds (Karim and Kamil., 1989; Pechsuth *et al*., 2001., Alam *et al*., 2003; Santos and Linardi, 2004; Rodríguez-Couto, 2009) Therefore, implementation of filamentous fungi for POME treatment could help ease the maintenance, monitoring and land requirement required by the conventional ponding system. Since typical ponding system requires longer hydraulic retention time where it involves higher operating costs, hence, by applying filamentous fungi to treat POME it is believed that the retention time required for treatment can be considerably reduced. Moreover, as compared to the conventional treatment method, it may offer better quality final effluent, less usage of chemicals, low energy consumption and low maintenance and operating costs since filamentous fungi can be easily obtained in nature.

Treatment of POME does generate greater amount of biosolids/ sludge thus proper management of the biosolids is vital in order to prevent loss of effective treatment capacity as well as to alleviate public health risks due to unsafe disposal. Since POME is non toxic therefore reuse of the biosolids for variety of applications are considered safe and would also be beneficial in terms of economic. Moreover, POME is rich with high biodegradable content of various suspended components of complex compounds such as proteins, fats, starch, cellulose, hemicelluloses, free organic acids and minor organic and mineral elements making them ideal substrates for production of fungal biomass protein. The fungal biomass protein could be converted into marketable products such as human and animal feed and may compensate the operating costs of the treatment process. Fortunately, the fungal biomass contains substantial quantities of nutrients such as carbohydrates, lipids, minerals and proteins thus making them also advantageous to be used as aquaculture feed ingredients. Furthermore, due to its high value proteins and fibres, fungal biomass could possibly be applied in the food sectors as human and animal foodstuffs replacing traditional plant or animal sources (Moore and Chiu, 2001).

In addition, kinetic studies play a major role in providing useful information either chemical or biological reactions progression occurred in a system (Jin *et al*., 2002). In this research, the study of biodegradation kinetic is important since it is helpful in describing how the filamentous fungi work and predicting its behavior in a system. Moreover, the kinetic coefficients which will be obtained in this study will be able to illustrate the biodegradation mechanism occurred. Besides, the kinetics study can be used to describe the relationship between theoretical states of variables and explains the biodegradation system performance (Pazaouki *et al*., 2008).

1.2 Problem statement

Filamentous fungi have already been applied to treat either domestic wastewater (Alam *et al*., 2003; Mannan *et al*., 2005) or industrial wastewater (Karim and Kamil., 1989; Pechsuth *et al*., 2001; Garcia *et al*., 2000). Nonetheless, application of filamentous fungi especially fungal genera of *Aspergillus* and *Trichoderma* on fresh POME biodegradation does receive lack of attention with less information published in the literatures. It is believed that the filamentous fungi exhibit strong capability to treat wastewater with very high organic loadings. Furthermore, the current method of ponding system for treatment of POME does have some drawbacks such as large of space requirement, long retention time of process treatment and also generates vast amount of odorous gas which may adversely affect the environment. Moreover, existing alternatives techniques for POME treatment based on physical and chemical treatments (Ahmad *et al*., 2005; Laohaprapanon *et al*., 2005; Agustin *et al*., 2008) involve high operating cost and expensive equipment. Realizing to this, an eco-friendly and low cost technique to treat fresh POME is crucial thus performing an investigation to study the effectiveness of the filamentous fungi on fresh POME biodegradation would be beneficial.

Both mixed and pure fungal cultures have been used successfully in the biodegradation of wastewater ingredients (Jianlong *et al*., 2001; Khalaf, 2008; Singh and Dikshit, 2010; Tsekova *et al*., 2010). The degradation of wastewater components has been investigated previously and it was concluded that most fungal species are excellent metabolizer (Okoro *et al*., 2008). As mentioned before, there are few researches on POME treatment by filamentous fungi thus establishement the role of filamentous fungi in POME degradation by exposing POME to pure fungal cultures is important. It is crucial to determine the pontential of pure culture in application for POME degradation before implementing the investigation using mixed cultures. In this study, the ability of two pure fungal cultures, *Aspergillus niger* and *Trichoderma virens* to degrade fresh POME are demonstrated.

Management of biosolids generated during POME treatment process plays an important role in preventing loss of effective treatment capacity due to sludge accumulation. Because POME is non toxic and contains large amounts of organic matters, macro and micro-nutrients therefore safe handling and use of the biosolids/ fungal biomass protein generated for human and animal consumption would be beneficial. Additionally, it is believed that filamentous fungi exhibit high potential ability to convert the ingredients present in the wastewater into valuable end products of fungal biomass protein which may enrich with appreciable amounts of protein and amino acids that can be used as a protein-rich animal feed. Moreover, the biomass produced by filamentous fungi can be used as such without any further processing because it provides carbohydrates, lipids, minerals, vitamins and proteins (Nitayavardhana and Khanal, 2010). Furthermore, it was reported that bioconversion of wastewater sludge by fungi into value added products such as organic acids, industrial enzymes, and, biopolymers have been completed with successful results therefore may able to compensate the operating costs of the treatment process (Barnab’e *et al*., 2009; More *et al*., 2010).

Nevertheless, to the best of our knowledge, there is lack of information on aerobic biodegradation of fresh POME by filamentous fungi as well as the kinetics data compared to the anaerobic digestion. Therefore, the kinetics study of the aerobic treatment of POME by filamentous fungi should be looked into and explored by determination of kinetic coefficients so that the biodegradation process can be understood. The knowledge of kinetic coefficients is necessary and useful for biological design control process as well as the optimization of the operational conditions (Nakhla *et al*., 2006). Moreover, by conducting kinetics study the relation between growth rate and substrate utilization could be estimated and would be beneficial for mass balance evaluation in a wastewater treatment plant (Firozjaee *et al*., 2011).

1.3 Objectives of the study

The main objective of the study is to compare the performance of two species of filamentous fungi namely *Aspergillus niger* and *Trichoderma virens* in fresh palm oil mill effluent (POME) biodegradation and fungal biomass protein recovery.

1.4 Scopes of the study

1. Evaluation of fresh POME degradation including total suspended solids (TSS), turbidity, specific resistance to filtration (SRF) and chemical oxygen demand (COD) removal rate.
2. Assessment of fungal biomass and protein recovery during treatment process
3. Optimization of biodegradation and fungal biomass protein recovery. Parameters chosen to be optimize are inoculums size (5-20%), agitation rate (100-150 rpm) and temperature (30-36^{o}C)
4. Determination of biodegradation kinetic coefficients (μ_{max}, K_s, $Y_{x/s}$) based on Monod’s model.

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