CO-CULTIVATION OF YEAST AND MICROALGAE ON POME FOR SIMULTANEOUS COD REDUCTION AND LIPID PRODUCTION

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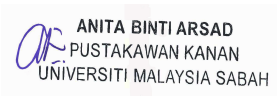

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I hereby declare that the material in this thesis is my own except for quotations, equations, summaries, and references, which have been duly acknowledged.

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ABSTRACT

Palm oil mill effluent (POME) contains high chemical oxygen demand (COD) which needs to be treated before discharge to waterbodies. Oleaginous yeast and microalgae have been known capable to grow and utilize nutrients present in POME resulting in COD reduction besides the benefits of microbial oils production as an alternative source for biodiesel. Thus, this study will focus on the cultivation of yeast (*Rhodotorula toruloides*) and microalgae (*Ankistrodesmus fusiformis*) on POME as a cheap culture medium to stimulate cell growth. It is hypothesized that utilizing POME as cheap substrate medium will effectively aid the cell growth of *R. toruloides* and *A. fusiformis*. Moreover, the co-cultivation of *R. toruloides* and *A. fusiformis* in POME will improve the COD reduction and lipid production as biodiesel feedstock via synergistic activity, as compared to a single culture. The optimum conditions [POME concentration (%), growth duration (days), and yeast to algae ratio] were optimized using Response Surface Methodology (RSM) through Design Expert Software (DOE) and analysed based on COD concentration (mg/L) lipid productivity (mg/ml/day), and biomass yield (mg/ml). Based on this study, co-cultivation in POME outperformed single culture with highest COD reduction efficiency and lipid production by 87.52% (4,235 mg/L) and 15.39 mg/ml/day respectively, whereas the COD reduction and lipid production by *R. toruloides* and *A. fusiformis* were 43%, 47%, and 4.51%, 1.67% correspondingly. Based on the analysis, all models showed significant result with p-value of $p<0.05$. In addition, the optimum conditions for COD removal and lipid production were 59.88% of POME concentration, 0.36 (9:25) yeast to microalgae ratio, and 18 days of growth duration. Thus, these findings show the utilization of POME as a cheap culture medium has enhanced the COD reduction and lipid production by co-cultivation as compared to single culture. In the future, the study in-depth based on the optimized condition of this co-culture would contribute to greater

COD reduction and lipid production by applying more alternative method such as application of a catalyst.

ABSTRAK

PENANAMAN YIS DAN MIKROALGAE DALAM POME UNTUK PEMBUANGAN SISA DAN PENGHASILAN MINYAK SECARA SERENTAK

Industri minyak sawit di Malaysia telah menimbulkan kebimbangan terhadap keselamatan alam sekitar. Efluen kilang kelapa sawit (POME) mengandungi permintaan oksigen kimia (COD) yang tinggi yang perlu dirawat sebelum dilepaskan ke saluran air. Oleaginous yis dan mickroalgae telah diketahui mampu tumbuh dengan menggunakan nutrien yang terdapat dalam POME dan mengakibatkan pengurangan COD selain manfaat dalam pengeluaran minyak mikrob sebagai sumber alternatif untuk biodiesel. Kajian ini akan memberi tumpuan kepada penanaman yis terpencil tempatan (Rhodotorula toruloides) dan mikroalgae (Ankistrodesmus fusiformis) dalam POME sebagai medium kultur murah untuk merangsang pertumbuhan sel. Berdasarkan hipotesis, penggunaan POME sebagai kultur pertumbuhan untuk R. toruloides dan A. fusiformis telah membantu perkembangan cell. Selain itu, pembinaan ko-kultur yis dan mikroalgae dalam POME akan meningkatkan pengurangan COD dan pengeluaran lipid sebagai biodiesel melalui aktiviti sinergi, berbanding dengan penanaman satu kultur. Keadaan optimum (kepekatan POME (%), tempoh pertumbuhan (hari), dan nisbah yis kepada alga) ditentukan daripada Metodologi Permukaan Tindak Balas (RSM) menggunakan Design Expert Software (DOE) dan dianalisis berdasarkan pengurangan COD (mg/L), produktiviti lipid (mg/ml/day), dan biojisim (mg/ml). Berdasarkan kajian ini, ko-kultur mempamerkan pengurangan COD dan lipid yang tertinggi berbanding dengan satu kultur iaitu 87.52% (4,235 mg/L*) dan 15.39 mg/ml/day manakala kadar pengurangan COD dan penghasilan lipid bagi R. toruloides dan A. fusiformis adalah seperti berikut 43%, 47%, dan 4.51%, 1.67%. Berdasarkan analisa kajian, model pengurangan COD dan penghasilan lipid menunjukkan keputusan yang signifikan dengan nilai p<0.05. Selain itu, kadar optimum untuk pengurangan COD dan penghasilan lipid adalah bedasarkan kepekatan POME sebanyak 59.88%, 0.36 (9:25)*

nisbah yis kepada mikroalga, dan 18 hari kadar pertumbuhan. Penemuan kajian ini dapat menunjukkan penggunaan POME sebagai media yang murah dapat membantu meningkatkan pengurangan COD dan penghasilan lipid oleh ko-kultur berbanding satu kultur. Pada masa yang akan datang, hasil daripada penyelidikan analisa yang telah dioptimis ini dapat menyumbangkan kepada pengurangan COD dan penghasilan lipid yang banyak dengan mengaplikasikan metodologi yang berbeza seperti pemangkin.

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LIST OF SYMBOLS

&	-	And
:	-	Ratio
+	-	Positive
-	-	Negative
^{0}C	-	Degree Celcius
%	-	Percentage
cm	-	Centimetre
µm	-	Micrometer
g	-	Gram
m	-	Meter
min	-	Minute
M	-	Molar
mL	-	millilitre
mg	-	milligram
h	-	Hour
d	-	Day
rpm	-	Revolution per minute
DW	-	Dry weight

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Palm oil industry has generated profitable products which has been utilized worldwide in biofuel, body care, and agri-food (Mutsaers *et al.*, 2019). The largest consumers of palm oil were known to be from Asia and Africa with the merge intake of 57 million tonnes substantially (Chew *et al.,* 2021). In Asia, after Indonesia, Malaysia has been appointed as the second ranked as top producer of crude palm oil in the World with 24% of total global crude palm oil production (Chea *et al.,* 2023).

Despite having its interest in wide industry, the faults in implementation of palm oil are indisputable. One of the biggest concerns are deforestation and high amount of land usage. Not just that, the palm oil products have often associated with peatland conversion, biomass waste creation, greenhouse gas pollution, and species extinction (Abideen *et al.,* 2023). Furthermore, by-products such as palm oil mill effluent (POME) resulting from the mill that was dispensed to watercourses has raised a concern over water safety. This is due to high content of chemical oxygen demand (COD) and biochemical oxygen demand (BOD) in POME which contribute to water pollution (Ooi *et al.,* 2023).

POME comprise with approximately 25000 ± 1000 mg/L of BOD and 2845 ± 159 mg/L of COD, 753 ± 15 mg/L of total nitrogen content, 102 ± 2 mg/L of phosphate and very acidic with pH values around 3 to 4 (Kumaran *et al.,* 2022). Thus, extensive research on developing alternatives approach in POME treatment prior to discharge to water bodies become crucial in combating environment degradation. According to Yashni *et al.* (2021), there are few known conventional method in treating POME such as biological, chemical, and physical type of treatment. The biological treatment usually affiliated with ponding system whereby implying the aerobic and anaerobic process that entails microorganisms such as bacteria, algae, yeast, and fungi for lipids degradation in POME. Besides that, the chemical and physical treatment involves chemical properties alteration and screening, sedimentation, and oil removal.

Out of many alternatives methods that has been developed for POME treatment, previous findings stated that aerobic and anaerobic biological processes have significantly cater in energy efficiency, low nutrient intake and biomass yield (Lee *et al.,* 2019). In Malaysia, there are more than 85% of POME producers has implied ponding system which involved in utilizing the anaerobic technology for POME treatment on the grounds that serve low cost for operation and capital (Noor *et al.,* 2021). Nonetheless, this method includes high retention time, low removal efficiency for some parameters, and requirement of large land area (Yashni *et al*., 2021).

Another viable approach for POME treatment by adopting oleaginous microorganism which has gained interest known from its characteristic for yielding lipid concurrently utilizing wastewater or POME as cheap culture medium for its growth (Karim *et al.,* 2021). Oleaginous microorganism defined as microbial are capable in producing lipid more than 25% which classified as biodiesel production and known to be as single cell oils (SCO). However, not all the microorganism identifies as biodiesel production (Wang *et al.,* 2021). Oleaginous microorganism can be derived from yeast, microalgae, fungi, and bacteria whereas they accrue more than 20 % w/w of lipid on a cell dry weight basis and has the capabilities of synthesizing prodigious fatty acids (Matsakas *et al.,* 2020). Application of oleaginous microorganisms in treating POME has gained favour globally

for potential replacement of vegetable oils and future biodiesel. Likewise, the culturing oleaginous microorganisms are more practical since it's not affected by seasons or climate change (Fakankun *et al.,* 2019).

Oleaginous yeast that are known to produce biodiesel are *Cutaneotrichosporon oleaginosus*, *Rhodotorula toruloides* and *Yarrowia lipolytica* (Abeln and Chuck, 2021). The implementation of oleaginous yeast in POME are beneficial in lipid production as well as a bioremediation which imply that the yeast takes up the rich organic compound in POME for its growth as well as treating wastewater (Islam *et al*., 2018). Yeast is more superior than the rest of the oleaginous microorganisms due to its capacity in utilizing low cost of fermentation media, exhibit short period of growth and cultivation in large scale (Patel *et al.,* 2020).

One of the known species is *Rhodotorula toruloides* of the *Pucciniomycotina* subph ylum that distinguished in cell growth rate of high-density fermentation, high carotenoid, and lipid productivity, and efficient in utilizing cheap substrate for its growth medium (Liu *et al*., 2018). Besides, based on data retrieved from National Centre for Biotechnology Information, the *R. toruloides* acclaimed to be exquisite microbial lipid producer that capable in producing over 70 % lipid of dry cell mass with a yield over 100 g/L. Cultivation of *Rhodotorula sp.* In POME has efficiently producing higher biomass yield in the state of high nitrogen source but less preferable in lipid production. Thus, this study chooses this strain for further investigate on behaviour in POME.

The production of biofuels from microalgae as the third generation of feedstock has gained the interest globally as one of the renewable biofuels and capable of reducing the green-house gas emissions (Chowdhury and Loganathan, 2019). Biofuels are produced from biological materials such as animals, plants, microorganisms, and wastes which has been use widely as a replacement from the unpractical old conventional method such as fossil fuels by having different method or process that distinctively functions based on "present-day" photosynthetic conversion of solar energy to chemical