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ORIGINAL LITERARY WORK DECLARATION

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ABSTRACT

The palm oil industry in Malaysia is one of the largest producers of agroindustrial wastewater known as Palm Oil Mill Effluent (POME). POME is highly organic in content which is acidic, thick, dark in colour, with high levels of chemical oxygen demand, ammonical nitrogen, orthophosphate, nitrate, nitrite, total suspended solids, and total solids. Due to high organic load, the raw POME has to be digested anaerobically to reduce the pollutant level, in order to meet the effluent discharge standard for palm oil mills. The objective of the present study is to investigate the potential of selected microalgae to grow in POME and anaerobic liquor (AL) as well as to assess the biochemical composition of the biomass and finally the percentage of pollution reduction obtained. Nine microalgae strains from the University of Malaya Algae Culture Collection (UMACC) which are Chlorella vulgaris UMACC 001, Scenedesmus UMACC 010, Scenedesmus UMACC 036, Scenedesmus UMACC 068, Ankistrodesmus convolutus UMACC 101, Nannochloris bacillaris UMACC 109, Chlorococcum oviforme UMACC 110, Chlamydomonas augustae UMACC 246 and Chlorella UMACC 300 were screened for growth and biochemical composition using flask cultures. Of nine strains, Chlorella UMACC 300 (specific growth rate (µ) =0.35±0.02 d⁻¹ and 39.88±2.73% DW of lipid), Chlorella vulgaris UMACC 001 (µ=0.32±0.02 and 40.61±2.78% DW of lipid), Scenedesmus UMACC 036 (µ=0.30±0.05 d⁻¹ and 32.75±1.01% DW of lipid) and Ankistrodesmus convolutus (µ=0.28±0.02 d⁻¹ and 38.89 ±2.70% DW of lipid) were selected to grow in different concentrations of POME and anaerobic liquor. The Chlorella UMACC 300 showed better tolerance to grow in higher concentrations of POME and AL. Chlorella UMACC 300 which grew in 25% AL (Bold Basal Medium, BBM) produce biomass of 677.33±11.37 mg L⁻¹ with 39.98±0.38 %DW protein, 19.12 ± 0.12 %DW carbohydrate and 43.44±0.92 %DW lipid on day 16 of culture period. This Chlorella UMACC 300
also produced higher pollution reduction when compared with other three selected microalgae, whereby it attained 87.66±0.59% reduction of chemical oxygen demand (COD), 65.38±2.51% ammonical nitrogen, 78.56±6.54% orthophosphate, 45.24±4.12 % nitrate and 42.13±11.81% nitrite. The second best strain which grew in different concentrations of POME and AL was Chlorella vulgaris UMACC 001 followed by Scenedesmus UMACC 036 and Ankistrodesmus convolutus respectively. The results obtained from this study shows that Chlorella UMACC 300 which was isolated from POME is tolerant of POME and AL and may be a potential species to be used for POME and AL treatment.
ABSTRAK

Industri kelapa sawit merupakan penyebab utama kepada bahan pencemar agroindustri yang dikenali sebagai efluen kilang kelapa sawit (POME). POME merupakan pencemar yang mempunyai kandungan organic yang tinggi dan bersifat asid, pekat, berwarna gelap dan mengandungi keupayaan kimia oksigen, ammonikal nitrogen, orthofosfat, nitrat, nitrit, total suspended solid and total solid. Disebabkan oleh kandungan organik yang tinggi, POME perlu dicerna secara anaerobik untuk mengurangkan kandungan bahan pencemar sebelum disalur keluar dari kilang pemprosesan minyak kelapa sawit, bagi memenuhi ‘Discharge Standards’ yang ditetapkan untuk kilang kelapa sawit. Objektif utama dalam kajian ini adalah untuk mengkaji potensi mikroalga terpilih untuk bertumbuh di media POME dan POME dicerna (AL) yang disediakan menggunakan kepekatan yang berbeza, bagi menilai kandungan biokimia and peratusan penurunan kandungan pencemar. Untuk itu, sembilan microalga dari ‘University Malaya Algae Culture Collection’ ia itu Chlorella vulgaris UMACC 001, Scenedesmus UMACC 010, Scenedesmus UMACC 036, Scenedesmus UMACC 068, Ankistrodesmus convolutus UMACC 101, Nannochloris bacillaris UMACC 109, Chlorococcum oviforme UMACC 110, Chlamydomonas augustae UMACC 246 and Chlorella UMACC 300 telah dikaji untuk mengenalpasti kandungan biokimia menggunakan sistem kultur. Daripada sembilan mikroalga Chlorella UMACC 300 (kadar pertumbuhan sesifik ($\mu$)=0.35±0.02d$^{-1}$ dan 39.88±2.73%DW lipid), Chlorella vulgaris UMACC 001($\mu$=0.32±0.02 dan 40.61±2.78%DW lipid), Scenedesmus UMACC 036 ($\mu$=0.30±0.05d$^{-1}$ dan 32.75±1.01%DW lipid) and Ankistrodesmus convolutus ($\mu$=0.28±0.02d$^{-1}$ dan 38.89±2.70%DW of lipid) telah dipilih untuk dikulturkan di dalam POME dan AL media yang disediakan menggunakan pelbagai kepekatan. Chlorella UMACC 300 menunjukkan toleransi yang tinggi untuk tumbuh di dalam POME dan AL yang berkepekatan tinggi. Chlorella UMACC 300 turut menunjukkan
pertumbuhan yang tinggi di dalam 25% AL (BBM) dengan 448.00±18.33mg L biomass, 27.70±0.93%DW protein, 1.60±0.07%DW karbohidrat dan 30.67±1.38%DW lipid. Mikroalga ini turut mencatatkan peratusan penurunan kandungan pencemar yang tinggi, dimana 87.66±0.59% penurunan COD, 65.38±2.51% ammonikal nitrogen, 78.56±6.54% orthofosfat, 45.24±4.12% nitrat and 42.13±11.81% nitrit. Mikroalga *Chlorella vulgaris* UMACC 001 menujukkan toleransi kedua tetinggi untuk bertumbuh di dalam POME dan di dalam AL. *Chlorella* UMACC 300 merupakan mikroalga yang mempunyai toleransi yang tinggi untuk bertumbuh di dalam POME media dan dapat mencapai peratusan penurunan pencemar yang tinggi. Keputusan yang diperolehi melalui kajian ini menunjukkan bahawa mikroalga yang berasal daripada POME mempunyai toleransi yang tinggi untuk bertumbuh di dalam POME dan di dalam AL, dimana mikroalga ini lebih sesuai untuk digunakan merawat POME dan AL.
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e) BBM medium (control)

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<tr>
<td>BBM</td>
<td>Bold’ Basal Medium</td>
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<tr>
<td>CDM</td>
<td>Clean Development Mechanism</td>
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<tr>
<td>CER</td>
<td>Certified Emission Reduction</td>
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<td>Chl-a</td>
<td>Chlorophyll-a</td>
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<tr>
<td>COD</td>
<td>Chemical Oxygen Demand</td>
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<tr>
<td>CPO</td>
<td>Crude Palm Oil</td>
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<td>DEE</td>
<td>Department of Environmental Enforcement</td>
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</tr>
<tr>
<td>DW</td>
<td>Dry Weight</td>
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<td>EQA</td>
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<tr>
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<td>FAME</td>
<td>Fatty Acid Methyl Esters</td>
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<tr>
<td>FFB</td>
<td>Fresh Fruit Bunch</td>
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<td>FGB</td>
<td>First Generation Biofuel</td>
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<tr>
<td>GC</td>
<td>Gas Chromatography</td>
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<td>GHG</td>
<td>Green House Gases</td>
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<td>GW</td>
<td>Global Warming</td>
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<td>HEPES</td>
<td>4-(2-Hydroxyethyl)-1-piperazinethanesulfonic acid</td>
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<td>HRAP</td>
<td>High Rate Algal Pond</td>
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</tr>
<tr>
<td>HRT</td>
<td>Hydraulic Retention Time</td>
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<td>IPCC</td>
<td>Intergovernmental Panel for Climate Changes</td>
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<tr>
<td>MAS</td>
<td>Membrane Anaerobic System</td>
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<tr>
<td>MUFA</td>
<td>Monounsaturated Fatty Acid</td>
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</table>
NaOH - Sodium hydroxide
OD - Optical Density
pH - Potential of hydrogen
PHA - Polyhydroxyalkanoates
POME - Palm Oil Mill Effluent
PUFA - Polyunsaturated Fatty Acid
RPM - Rotates per Minute
SBR - Sequencing Batch Reactor
SFA - Saturated Fatty Acid
SGB - Second Generation Biofuel
STR - Solid Retention Time
TDS - Total Dissolved Solid
TGB - Third Generation Biofuel
TS - Total Solid
TSS - Total Suspended Solid
TVS - Total Volatile Solid
UMACC - University of Malaya Algae Culture Collection
# LIST OF SYMBOL AND UNITS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tr>
<td>%</td>
<td>percent</td>
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<tr>
<td>µ</td>
<td>specific growth rate</td>
</tr>
<tr>
<td>µg</td>
<td>micro gram</td>
</tr>
<tr>
<td>cell / mL</td>
<td>cells per millimetre</td>
</tr>
<tr>
<td>d⁻¹</td>
<td>per day</td>
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<tr>
<td>g</td>
<td>gram</td>
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<tr>
<td>mg / L</td>
<td>milligram per liter</td>
</tr>
<tr>
<td>ºC</td>
<td>degree Celcius</td>
</tr>
<tr>
<td>OD₆₂₀nm</td>
<td>Optical density at 620nm</td>
</tr>
<tr>
<td>psi</td>
<td>pound per square inch</td>
</tr>
<tr>
<td>Rpm</td>
<td>revolution per minute</td>
</tr>
<tr>
<td>v/v</td>
<td>volume per volume</td>
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<tr>
<td>w/v</td>
<td>weight per volume</td>
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</table>
### LIST OF APPENDICES

**Appendix A:** OD$_{620\text{nm}}$ of nine microalgae strains culture on BBM medium for 12 days

**Appendix B:** Cell Count of Microalgae Cultured in BBM Medium for 12 Days of Culture Period

1. *Chlorella vulgaris* UMACC 001
2. *Scenedesmus* UMACC 010
3. *Scenedesmus* UMACC 036
4. *Scenedesmus* UMACC 068
5. *Ankistrodesmus convolutus* UMACC 101
6. *Nannochloris bacillaris* UMACC 109
7. *Chlorococcum oviforme* UMACC 110
8. *Chlamydomonas augustae* UMACC 246
9. *Chlorella* UMACC 300

**Appendix C:** Chlorophyll –a content of Microalgae Cultured in BBM Medium for 12 Days of Culture Period

1. *Chlorella vulgaris* UMACC 001
2. *Scenedesmus* UMACC 010
3. *Scenedesmus* UMACC 036
4. *Scenedesmus* UMACC 068
5. *Ankistrodesmus convolutus* UMACC 101
6. *Nannochloris bacillaris* UMACC 109
7. *Chlorococcum oviforme* UMACC 110
8. *Chlamydomonas augustae* UMACC 246
9. *Chlorella* UMACC 300

**Appendix D:** Carotenoid content of Microalgae Cultured in BBM Medium for 12 Days of Culture Period

1. *Chlorella vulgaris* UMACC 001
2. *Scenedesmus* UMACC 010
3. *Scenedesmus* UMACC 036
4. *Scenedesmus* UMACC 068
5. *Ankistrodesmus convolutus* UMACC 101
6. *Nannochloris bacillaris* UMACC 109
7. *Chlorococcum oviforme* UMACC 110
8. *Chlamydomonas augustae* UMACC 246
9. *Chlorella* UMACC 300
### Appendix E: Specific Growth Rate (µ) Based on Chlorophyll-a Content of Microalgae Cultured in BBM Medium for 12 Days of Culture Period

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### Appendix F: Biomass Content of Microalgae Cultured in BBM Medium for 12 Days of Culture Period

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<th>Microalgae</th>
<th>Culture Medium</th>
<th>UMACC Code</th>
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<tbody>
<tr>
<td><strong>I. Chlorella vulgaris</strong> UMACC 001</td>
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<td><strong>II. Scenedesmus</strong> UMACC 010</td>
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<td><strong>IV. Scenedesmus</strong> UMACC 068</td>
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<td><strong>V. Ankistrodesmus convolutus</strong> UMACC 101</td>
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<td><strong>VI. Nannochloris bacillaris</strong> UMACC 109</td>
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<td><strong>VII. Chlorococcum oviforme</strong> UMACC110</td>
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<td></td>
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<tr>
<td><strong>VIII. Chlamydomonas augustae</strong> UMACC 246</td>
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<td><strong>IX. Chlorella</strong> UMACC 300</td>
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### Appendix G: Protein Content of Microalgae Cultured in BBM Medium for 12 Days of Culture Period

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<td><strong>IV. Scenedesmus</strong> UMACC 068</td>
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<tr>
<td><strong>V. Ankistrodesmus convolutus</strong> UMACC 101</td>
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<td><strong>VI. Nannochloris bacillaris</strong> UMACC 109</td>
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<tr>
<td><strong>VII. Chlorococcum oviforme</strong> UMACC110</td>
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<tr>
<td><strong>VIII. Chlamydomonas augustae</strong> UMACC 246</td>
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<td><strong>IX. Chlorella</strong> UMACC 300</td>
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### Appendix H: Carbohydrate Content of Microalgae Cultured in BBM Medium for 12 Days of Culture Period

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<th>Microalgae</th>
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<td><strong>V. Ankistrodesmus convolutus</strong> UMACC 101</td>
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<td><strong>VI. Nannochloris bacillaris</strong> UMACC 109</td>
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<td><strong>VIII. Chlamydomonas augustae</strong> UMACC 246</td>
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<td><strong>IX. Chlorella</strong> UMACC 300</td>
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### Appendix I: Lipid Content of Microalgae Cultured in BBM Medium for 12 Days of Culture Period

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IV. *Scenedesmus* UMACC 068
V. *Ankistrodesmus convolutus* UMACC 101
VI. *Nannochloris bacillaris* UMACC 109
VII. *Chlorococcum oviforme* UMACC110
IX. *Chlorella* UMACC 300

Appendix J: Fatty Acid Composition of Microalgae Cultured in BBM Medium for 12 Days of Culture Period
   I. *Chlorella vulgaris* UMACC 001
   II. *Scenedesmus* UMACC 010
   III. *Scenedesmus* UMACC 036
   IV. *Scenedesmus* UMACC 068
   V. *Ankistrodesmus convolutus* UMACC 101
   VI. *Nannochloris bacillaris* UMACC 109
   VII. *Chlorococcum oviforme* UMACC110
   IX. *Chlorella* UMACC 300

Appendix K: Cell Count of Microalgae Cultured in Different Concentration of POME and AL
   I. *Chlorella* UMACC 300
   II. *Chlorella vulgaris* UMACC 001
   III. *Scenedesmus* UMACC 036
   IV. *Ankistrodesmus convolutus* UMACC 101

Appendix L: Specific Growth Rate($\mu$) Based on Cell Count of Microalgae Cultured in Different Concentration of POME and AL
   I. *Chlorella* UMACC 300
   II. *Chlorella vulgaris* UMACC 001
   III. *Scenedesmus* UMACC 036
   IV. *Ankistrodesmus convolutus* UMACC 101

Appendix M: Dry weight of Microalgae Cultured in Different Concentration of POME and AL on Day 0 and day 16
   I. *Chlorella* UMACC 300
   II. *Chlorella vulgaris* UMACC 001
   III. *Scenedesmus* UMACC 036
   IV. *Ankistrodesmus convolutus* UMACC 101
Appendix N: Protein Content Microalgae Cultured in Different Concentration of POME and AL on day 0 and Day 16
I. Chlorella UMACC 300
II. Chlorella vulgaris UMACC 001
III. Scenedesmus UMACC 036
IV. Ankistrodesmus convolutus UMACC 101

Appendix O: Carbohydrate Content of Microalgae Cultured in Different Concentration of POME and AL on Day 0 and Day 16
I. Chlorella UMACC 300
II. Chlorella vulgaris UMACC 001
III. Scenedesmus UMACC 036
IV. Ankistrodesmus convolutus UMACC 101

Appendix P: Lipid Content of Microalgae Cultured in Different Concentration of POME and AL on Day 0 and Day 16
I. Chlorella UMACC 300
II. Chlorella vulgaris UMACC 001
III. Scenedesmus UMACC 036
IV. Ankistrodesmus convolutus UMACC 101

Appendix Q: Fatty Acid Composition of Four Selected Microalgae Cultured in Different Concentration of POME and AL on day 16

A) Chlorella UMACC 300
I. 25% AL(distilled water)
II. 25% AL(BBM)
III. 25% Raw POME (distilled water)
IV. 50% AL (distilled water)
V. BBM(control)

(B) Chlorella vulgaris UMACC 001
I. 25% AL(distilled water)
II. 25% AL(BBM)
III. 25% Raw POME (distilled water)
IV. 50% AL (distilled water)
V. BBM(control)

(C) Scenedesmus UMACC 036
I. 25% AL(distilled water)
II. 25% AL(BBM)
III. 25% Raw POME (distilled water)
IV. 50% AL (distilled water)
V. BBM(control)
(D) *Ankistrodesmus convolutus* UMACC 101

I. 25% AL (distilled water)
II. 25% AL (BBM)
III. 25% Raw POME (distilled water)
IV. 50% AL (distilled water)
V. BBM (control)

Appendix R: Pollution Reduction profile of Microalgae Cultured in Different Concentration of POME and AL

A. *Chlorella* UMACC 300
   I. Chemical Oxygen Demand
   II. Orthophosphate
   III. Ammonical Nitrogen
   IV. Nitrate
   V. Nitrite

B. *Chlorella vulgaris* UMACC 001
   I. Chemical Oxygen Demand
   II. Orthophosphate
   III. Ammonical Nitrogen
   IV. Nitrate
   V. Nitrite

C. *Scenedesmus* UMACC 036
   I. Chemical Oxygen Demand
   II. Orthophosphate
   III. Ammonical Nitrogen
   IV. Nitrate
   V. Nitrite

D. *Ankistrodesmus convolutus* UMACC 101
   I. Chemical Oxygen Demand
   II. Orthophosphate
   III. Ammonical Nitrogen
   IV. Nitrate
   V. Nitrite

Appendix S: Characteristics of raw POME and Anaerobically digested POME (AL) collected from Tennamaram Estate Palm Oil Processing Mill to culture selected microalgae

I. *Chlorella* UMACC 300
II. *Chlorella vulgaris* UMACC 001
III. *Scenedesmus* UMACC 036
IV. *Ankistrodesmus convolutus* UMACC 101

300

320