Effect of Light/Dark cycle and carbon source on Lipid Production by Chlorella pyrenoidosa using Agro Waste Water Malaysia

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1. Background information
2. Research gap
3. Research Methodology
4. Result & Discussion
5. Conclusion
Overview of palm oil mill

1 tonne of fresh fruit bunches (FFB)

66 million tonnes/year

0.73 tonne of steam

0.18-0.22 tonne crude palm oil (CPO)
12.0 million tonnes

Waste
- 0.6-0.7 tonne POME (Liquid)
  47 million m³
- 0.19 tonne fibers + shells
- 0.23 tonne empty fruit bunches

(Solid) 27 mil. tonnes

Source: MPOB:2004
Source of Palm Oil Mill Effluent (POME)

Discharged as palm oil mill effluent

Source: Biomethane Production from Palm Oil Mill Effluent (Pome) in a Semi–commercial Closed Anaerobic Digester
<table>
<thead>
<tr>
<th>No</th>
<th>Parameter*</th>
<th>Concentration range</th>
<th>Malaysia Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(mg/L)</td>
<td>(mg/L)</td>
</tr>
<tr>
<td>1</td>
<td>pH</td>
<td>4.15 – 4.4.5</td>
<td>6.0 – 9.0</td>
</tr>
<tr>
<td>2</td>
<td>COD</td>
<td>1,350 – 2,120</td>
<td>50-100</td>
</tr>
<tr>
<td>3</td>
<td>Soluble COD</td>
<td>20,500 – 24,500</td>
<td>------</td>
</tr>
<tr>
<td>4</td>
<td>BOD</td>
<td>300 – 400</td>
<td>20-50</td>
</tr>
<tr>
<td>5</td>
<td>Total volatile solid</td>
<td>27,300 – 30,150</td>
<td>100-200</td>
</tr>
<tr>
<td>6</td>
<td>Total Suspended Solid</td>
<td>15,660 – 23,560</td>
<td>50-100</td>
</tr>
<tr>
<td>7</td>
<td>Total phosphorus</td>
<td>200 – 600</td>
<td>------</td>
</tr>
<tr>
<td>8</td>
<td>Total nitrogen</td>
<td>500 – 800</td>
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</tbody>
</table>

Can cause significant environmental effects like oxygen depletion if it is discharged without efficient treatment.
Conventional treatment method

- **Anaerobic treatment:**
  Long retention time, large area required.

- **Aerobic:**
  High energy requirement.

- **Membrane:**
  Short membrane life, membrane fouling, expensive.

- **Evaporation:**
  High energy consumption.
Concept of integrated palm oil based bio-refinery

- Harvesting
- Crushing
- Refining
- Biogas fuel
- Plastics
- Chemicals
- Fertilizers
- EFB
- Various routes
- Chemicals
- Fuels
- Biocomposites
- POME
- Fermentation
- Gasification
- Others
- Residues and products
- Final products
- Intermediate streams
- Refined
- Glycerine
- Fuels
- Chemicals
- Coatings
- Adhesives
- Foams
- Foods
- Soaps
- Cosmetics
- Other???
Environmental stress such as nutrient level and light intensity induces the growth of algae.

POME is a source of enormous organic and nutrient content, considered favorable for the growth of several types of microalgal species to produce lipid.
Microalgae are the primary producers of oxygen on the planet, generating 40–50% of the world’s atmospheric oxygen.

30,000 known species of microalgae and more are being discovered and categorized, however only a few hundred are actively being investigated and very few produce industrially.

Depending on species, microalgae produce many different kinds of lipids, hydrocarbons and protein.
Inhibit the microalgae growth

Waste water treatment

Promoting the microalgae growth

Waste-to-wealth opportunities
Faster grow
CO2 Capture
High lipid & fatty acid
Use saline, brackish, waste waters, seawater
Do not compete with feed crops
Wastewater treatment
Oil from algae is 7–31 times greater than palm oil

Sources: (Lam and Lee, 2011).
LITERATURE

Most abundant microalgae studied

1. diatoms (Bacillariophyceae)
2. green algae (Chlorophyceae)
3. blue-green algae (Cyanophyceae)
4. golden algae (Chrysophyceae)

Chlorella pyrenoidosa
COMPONENT OF TYPICAL MICROALGAE

Microalgae
- Proteins
- Carbohydrates
- Lipids
- Valuable compounds

Lipids
- Storage lipids
  - Mainly TAGs
  - Up to 50% of DW
  - With solvents extractable from wet biomass
- Membrane lipids
  - Different lipid classes
  - Up to 40% lipids are PUFA
  - Solubilised by solvent extraction of wet biomass, then transesterification

Research review

- Most studies reported that under intermittent illumination, higher specific light absorption together with the lower specific growth rate most probably contribute to the reduction of the biomass production of *C. pyrenoidosa*.

- However, with *C. reinhardtii*, the amount of chlorophyll a content was doubled under the intermittent illumination in comparison to continuous illumination. Due to the increase of chlorophyll a content under light/dark cycles, and lead to the increase of its specific light absorption. (Janssen et al., 1999)

- Whether the continuous illumination is beneficial to promote the growth of biomass and/or chlorophyll a, it could be varied based on specific strain of microalgae and possibly the combination of operating conditions including growth medium.
To evaluate the growth of microalgae species, i.e., *Chlorella pyrenoidosa*, a locally isolated species in POME

To evaluate effect of light regime (photo period) and C/N ratio on the biomass and lipid production
Culture medium and source of POME

- *C. pyrenoidosa* was isolated from a POME pond located in Johor Palm Oil Mill, Kahang, Johor, Malaysia.

- Cultivated and maintained in a 10-fold diluted POME provided with cultural conditions of 24°C - 26°C, with photoperiod of 8h:16h (in Light: in Dark), pH ranging between 6.5 - 7.5, and light intensity of 150 mol m² s⁻¹.

- POME was diluted 10 times to reduce the shadings effect on the microalgae growth. The growth of *C. pyrenoidosa* was assessed by optical density (OD) at a wavelength of 600 nm, chlorophyll content.
RESEARCH METHODOLOGY

Chlorella + BBM + POME

- BBM and Chlorella
- 250 mg COD/L of POME and BBM with Chlorella
- 500 mg COD/L POME and BBM with Chlorella
- 1000 mg COD/L POME and BBM with Chlorella
To characterize the produced lipid content from biomass using conventional techniques in order to find lipid Production

1. Expand cultivation of *chlorella*
2. Cultivate algae individually in raw POME with different dilution and light regime
3. Harvesting biomass using conventional techniques, mainly centrifuge
RESULTS AND DISCUSSION

Effect of photo period for the production of biomass and lipid by *Chlorella Pyrenoidosa* in POME

Lipid production

- L:D = (24:0) 17.00 mg/mg CDW
- L:D = (16:8) 13.10 mg/mg CDW
- L:D = (8:16) 9.43 mg/mg CDW

Specific growth rate (per day)

Time (d)
RESULTS AND DISCUSSION

- Organic Carbon Substrate and Nutrient Utilization Rate *via C. pyrenoidosa* from Settled Raw Palm Oil Mill Effluent (POME) for Lipid Production

![Graph showing organic carbon and nutrient consumption rates over time](image)

C:TN 100:6 shows the higher utilization rate.
CONCLUSION

- The highest lipid content achieved when *C. pyrenoidosa* cultured under continuous light (24 hr) at 18.00 mg lipid/mg CDW.

- In conclusion, *C. pyrenoidosa* was found to produce highest lipid when grown under C:TN ratio of 100:6, under continuous illumination and with OLR of 36 kg COD/m³.d.
THANK YOU

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