Enhanced photosynthetic growth, biodiesel and electricity production using *C. vulgaris* and *P. pudita*S. cerevisiae

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Introduction

- Decreasing supplies & increasing costs of existing energy sources – need alternative energy sources

- Canada: the 7th largest greenhouse gas emitter (per capita basis), by emitting about 747 megatonnes (79% CO₂) annually (Environment Canada, 2008)

- Reduction of emissions by sequestration & production of alternative biofuels are desirable

- Microbial Fuel Cell (MFC) generates electrical current
Introduction

Why Microalgae?

- A potential alternative energy source
- Photosynthesize CO$_2$
- Up to half their mass is made up of natural oils
- Algal 'crude', or refined to higher-grade hydrocarbon products, ranging from biodiesel to bio-jet fuel for aircraft
- Grow anywhere with less nutrients
- Algae yield 100 tonnes/ha and 2.2 tonnes CO$_2$ needed/1 tonnes algae (from coal-fired power stations)
Introduction

Why *Chlorella vulgaris*?

A great potential biodiesel source due to:
— high oil production capacity per unit cultivation area
— fast growth rate and low-cost culture conditions
— Tolerance for 0.03 to 40% CO$_2$
— High photosynthetic efficiency

AND

The same micro-algal cultures - used as a promising source for production of electrical energy in a MFC

Photomicrograph: *Chlorella vulgaris*
Introduction

The growth rate of *C. vulgaris* and its oil content depend on environmental factors such as
— light intensity
— growth media
— CO$_2$ supplied

AND

The economic feasibility of *C. vulgaris* culture for biodiesel production greatly depends on high biomass productivity and appreciable oil yields

SO

Optimization of these factors is needed to maximize biodiesel production and to increase its production rate

AND

The biodiesel content in *C. vulgaris* can be increased by controlling growth conditions in an ELAPB
Introduction

Microbial Fuel Cell (MFC)

- A microbial fuel cell is a system that recovers electrons produced during microbial metabolism and channels them to generate electrical current.
- A wide variety of MFC designs and microbial species have been studied for generation of electrical flow.
- A lot of fundamental work needs to be achieved in order to develop a ready-to-use technology for commercial applications.
Objectives

- To investigate the photosynthetic growth kinetics of *C. vulgaris* in a novel circulating loop photobioreactor

- To maximize biodiesel yield

- To produce electricity by developing a *C. vulgaris-P. pudita/ C. vulgaris-S. cerevisiae* MFC.
Materials and Methods

Cultures

*Chlorella vulgaris*

*Saccharomyces cerevisiae*

*Pseudomonas putida*
Materials and Methods

A Novel External Loop Airlift Photobioreactor (ELAPB)

Schematic of ELAPB (Sasi, D., 2009). Riser (1), Downcomer (2), Gas disengagement zone (3), Gas sparger (4), Flow meter (5)
Materials and Methods

Specification of ELAPB

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bioreactor loop length</td>
<td>3.058 m</td>
</tr>
<tr>
<td>Total circulation time</td>
<td>26.5 s</td>
</tr>
<tr>
<td>Average circulation velocity</td>
<td>0.12 m/s</td>
</tr>
<tr>
<td>Inner diameter of riser</td>
<td>50.8 mm</td>
</tr>
<tr>
<td>Inner diameter of downcomer</td>
<td>38.1 mm</td>
</tr>
<tr>
<td>Working volume</td>
<td>4.5 L</td>
</tr>
<tr>
<td>Number of orifices in sparger</td>
<td>12</td>
</tr>
<tr>
<td>Material</td>
<td>Acrylic</td>
</tr>
</tbody>
</table>
Materials and Methods

Algae cultivation and biodiesel yield

- Media feed
- Algae inoculation
- Photobioreactor (ELAPB)
- 
  - CO₂ & Air flow
  - Lights (LED)
- Dry algae biomass
- Centrifugation
- Vacuum dry
- Extraction
- Soxhlet
- Evaporation
- Raw oil
- Vacuum dry
Materials and Methods

Soxhlet Extraction: Experimental Set Up

Soxhlet Extractor

Rotary Evaporator
Materials and Methods

Biomass and Growth Rate Determination

Optical Density (OD): OD of biomass determined on a spectrophotometer (Shimadzu Corporation, Japan) at a wavelength of 620 nm.

Cell concentration (dry weight, mg/L) = OD* Conversion factor of Shimadzu spectrophotometer
Materials and Methods

**CO₂ source**

**Cathode (algae)**

**Light source**

**Anode (yeast/bacteria)**

**NAFION 112 membrane**

**Algae media feeding pump**

**Anode media feeding pump**

**Resistance box**

**Volt meter**

MFC: Original Laboratory Set Up
Schematic of electron flow in the completely biological MFC: (a) anodic release of electrons by consuming organic compounds, (b) cathodic capture of electrons by photosynthetic growth on CO$_2$ (Powell et al., 2009).
### Material and Methods

#### Specifications of the Complete MFC

<table>
<thead>
<tr>
<th>Specifications</th>
<th>Cathode</th>
<th>Bridging</th>
<th>Anode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working volume of the vessel</td>
<td>1.5 L</td>
<td></td>
<td>350 mL</td>
</tr>
<tr>
<td>Culture</td>
<td><em>C. vulgaris</em></td>
<td></td>
<td><em>P. pudita/S. cerevisiae</em></td>
</tr>
<tr>
<td>Media</td>
<td>Bold’s</td>
<td>Cathode and anode were linked by a NAFION 112 membrane for proton exchange</td>
<td>1 dose McKinney’s/yeast media</td>
</tr>
<tr>
<td>Air flow</td>
<td>190 mL/min</td>
<td>25 mL/min</td>
<td></td>
</tr>
<tr>
<td>CO₂ flow</td>
<td>10 mL/min</td>
<td>-</td>
<td>10 mg/L</td>
</tr>
<tr>
<td>Mediator (methylene blue)</td>
<td>5 mg/L</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Lights</td>
<td>2 GE 26 W helical fluorescent bulbs</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Media feeding rate</td>
<td>15 mL/h</td>
<td>35 mL/h</td>
<td></td>
</tr>
<tr>
<td>Electrode</td>
<td>Carbon rod</td>
<td>Carbon rod</td>
<td></td>
</tr>
</tbody>
</table>
Materials and Methods

Modeling

Applied loading resistances: 10000, 8000, 5000, 2000, 1000 & 250 Ω

First order empirical equation represents dynamic response:

\[ V_{\text{model}} = V_{\text{final}} - (V_{\text{final}} - V_{\text{initial}}) \times e^{-kt} \]

Where,
- \( V_{\text{initial}} \) = voltage (mV) recorded for the starting point of loading or unloading
- \( V_{\text{final}} \) = voltage (mV) recorded for the end point of loading and unloading
- \( k \) = rate constant (min\(^{-1}\))
- \( t \) = elapsed time (min)
Results and Discussion

Growth Kinetics of *C. vulgaris* and Biodiesel Yield

**Input variables (growth factors)**
- Media
- Lights
- Carbon dioxide
- Dark phase duration

**Output**
- Growth rate (h\(^{-1}\)) of *C. vulgaris*
- Biodiesel yield
Results and Discussion (on ELAPB)

The Growth kinetics of *Chlorella vulgaris* in ELAPB

**Fig. 1** Growth kinetics of *C. vulgaris* at highest growth rate.

**Fig. 2** Growth kinetics of *C. vulgaris* at lowest growth rate.
## Results and Discussion (on ELAPB)

### Table 1 Experimental conditions, growth rates and oil yields

<table>
<thead>
<tr>
<th>Run No.</th>
<th>CO₂ conc. (%)</th>
<th>Lights around Riser</th>
<th>Lights around Downcomer</th>
<th>Dark phase (hr.)</th>
<th>Media</th>
<th>Growth rate (hr⁻¹)</th>
<th>Oil yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>6</td>
<td>-</td>
<td>8</td>
<td>Bold’s</td>
<td>0.04</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>6</td>
<td>-</td>
<td>8</td>
<td>NaNO₃</td>
<td>0.05</td>
<td>4.5</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>6</td>
<td>-</td>
<td>8</td>
<td>2ppm FeCl₃</td>
<td>0.04</td>
<td>4.5</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>16</td>
<td>10</td>
<td>-</td>
<td>Bold’s</td>
<td>0.01</td>
<td>33</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>16</td>
<td>10</td>
<td>8</td>
<td>Bold’s</td>
<td>0.03</td>
<td>26</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>16</td>
<td>5</td>
<td>-</td>
<td>Bold’s</td>
<td>0.04</td>
<td>18</td>
</tr>
</tbody>
</table>
Results and discussion (on Algae-Bacteria MFC)

Electricity Generation in an Algae-Bacteria MFC

<table>
<thead>
<tr>
<th>Input</th>
<th>Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistances: 10000, 8000, 5000, 2000, 1000 and 250 Ω</td>
<td>Resistance effect on voltage</td>
</tr>
<tr>
<td></td>
<td>Response time and voltage history of the model</td>
</tr>
</tbody>
</table>
Results and Discussion (on Algae-Bacteria MFC)

Loading and Unloading Effect on the Circuit

**Fig. 3** Effect of resistance (10000 Ω)

**Fig. 4** Effect of resistance (8000 Ω)
Results and Discussion (on Algae-Bacteria MFC)

Loading and Unloading Effect on the Circuit

**Fig. 5** Effect of resistance (5000 Ω)

**Fig. 6** Effect of resistance (2000 Ω)
Results and Discussion (on Algae-Bacteria MFC)

Loading and Unloading Effect on the Circuit

**Fig. 7** Effect of resistance (1000 Ω)

**Fig. 8** Effect of resistance (250 Ω)
Results and Discussion (on Algae-Bacteria MFC)

Table 2 Voltage and response time for loading and unloading resistance (algae – bacteria MFC)

<table>
<thead>
<tr>
<th>Voltage (mV)</th>
<th>Loading Resistance</th>
<th>10000Ω</th>
<th>8000Ω</th>
<th>5000Ω</th>
<th>2000Ω</th>
<th>1000Ω</th>
<th>250Ω</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>-28</td>
<td>-40</td>
<td>-36.5</td>
<td>-24</td>
<td>-8.3</td>
<td>-55.5</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>-91</td>
<td>-82.9</td>
<td>-52.3</td>
<td>-20</td>
<td>-12.3</td>
<td>-128.8</td>
</tr>
<tr>
<td>Unloading</td>
<td>Initial</td>
<td>91</td>
<td>-83.5</td>
<td>-55.6</td>
<td>-25</td>
<td>-11.3</td>
<td>-127.8</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>67.3</td>
<td>77.8</td>
<td>77</td>
<td>102.7</td>
<td>72</td>
<td>44</td>
</tr>
<tr>
<td>Response time (min)</td>
<td>Loading</td>
<td>12.3</td>
<td>20</td>
<td>27.8</td>
<td>58.4</td>
<td>21.5</td>
<td>24.5</td>
</tr>
<tr>
<td></td>
<td>Unloading</td>
<td>75.3</td>
<td>83.7</td>
<td>78.7</td>
<td>81</td>
<td>59.5</td>
<td>75.3</td>
</tr>
</tbody>
</table>
Results and Discussion (on Algae-Yeast MFC)

Loading and Unloading Effect on the Circuit

Fig. 9 Effect of resistance (10000 Ω)  Fig. 10 Effect of resistance (8000 Ω)
Results and Discussion (on Algae-Yeast MFC)

Loading and Unloading Effect on the Circuit

Fig. 11 Effect of resistance (5000 Ω)  Fig. 12 Effect of resistance (2000 Ω)
Results and Discussion (on Algae-Yeast MFC)

Loading and Unloading Effect on the Circuit

**Fig. 13** Effect of resistance (1000 Ω)

**Fig. 14** Effect of resistance (250 Ω)
# Results and Discussion (on Algae-Yeast MFC)

**Table 3 Voltage and response time for loading and unloading resistance (algae – yeast MFC)**

| Voltage (mV) | Loading | | | | | | Loading Resistance |
|--------------|---------|---|---|---|---|---|
|              | Initial | 10000Ω | 8000Ω | 5000Ω | 2000Ω | 1000Ω | 250Ω |
| Voltage      |         |         |         |         |         |        |      |
| Initial      | 224     | 233     | 234     | 284     | 300     | 260    |
| Final        | 56      | 51.3    | 36.5    | 16.3    | 8       | 2.75   |
| Unloading    | initial |         |         |         |         |        |      |
| initial      | 56      | 53      | 36      | 16      | 8       | 2.75   |
| Final        | 227     | 232     | 221     | 281     | 278     | 261    |
| Response time (min) | Loading |         |         |         |         |        |      |
|               | 2.0     | 3.0     | 0.5     | 1.0     | 4.5     | 0.4    |
|               | Unloading | 25.5 | 29.0 | 15.0 | 20.5 | 19.5 | 18.5 |
Conclusions & Recommendations

- ELAB Photobioreactor can enhance photosynthetic algae growth by a factor of 5 compared to well-mixed bioreactors.

- Ideal light conditions cause high oil yields, as much as 35% of dry weight.

- Electricity was generated by constructing an algae-bacteria/yeast based MFC. The experimental potential differences and response times were effectively modeled to predict the effect of voltage drops and response times.

- Higher photosynthetic productivity now needs to be achieved using cell recycle (high biomass) and high electricity production.
Acknowledgements

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Thank You & Questions?