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APPENDICES

Appendix A Chemical Composition of *Scenedesmus armatus*

On ash-free basis, Milner (1953) proposed two simultaneous equations to determine the chemical composition of algae:

$$42(\%P) + 28(\%C) + 67.5\ (\%L) = (R\text{-value})(100\%) \qquad (A1)$$

$$\%P + \%C + \%L = 100\% \qquad (A2)$$

where P, C, and L signify protein, carbohydrate, and lipid, respectively. R-value expresses the degree of reduction of the total content of organic matter in algae which is proportional to the heat of combustion of the algae. The scale of R-value runs from 0 to 100. Since freshwater algae have high energy content, so R-value is quite high as well. For *Scenedesmus armatus*, R-value is assumed to be 50.

Rodjaroen *et al.* (2007) reported carbohydrate content of 25 native strains of microalgae in Thailand, including *Scenedesmus armatus*. The average carbohydrate content of *Scenedesmus armatus* is 15 % dry weight.

Next, substitute R-value into Eq. (A1) and carbohydrate content (%C) into Eq. (A1) and (A2). Then solve Eq. (A1) and (A2) simultaneously to calculate protein and lipid content of *Scenedesmus armatus*. The result of chemical composition of *Scenedesmus armatus* is shown in Table A1.

Table A1 Chemical composition of *Scenedesmus armatus*

Composition	Protein	Carbohydrate	Lipid
Content (% ash-free dry weight)	45	15	40

It is interesting to note that the result from this calculation is closed to Oilgae (2011) who reported that the lipid content of *Scenedesmus* species is 45 % dry weight. This comparison can be used to guarantee that the calculation result is practical to use in this study.

Appendix B Chemical Reaction Balance

B1 Sodium nitrate ($NaNO_3$)

$$2HNO_3 + Na_2CO_3 \rightarrow 2NaNO_3 + H_2O + CO_2$$

	Reactants		Products		
	HNO_3	Na_2CO_3	$NaNO_3$	H_2O	CO_2
Molecular weight (g/mol)	63.013	105.988	84.9945	18.015	44.01
Mass (g)	126.026	105.988	169.989	18.015	44.01

Mass allocation: Total mass = 169.989 + 18.015 + 44.01 = 232.014 g

$$NaNO_3 = \frac{169.989}{232.014} \times 100 = 73.3\ \%$$

$$H_2O = \frac{18.015}{232.014} \times 100 = 7.8\ \%$$

$$CO_2 = \frac{44.01}{232.014} \times 100 = 18.9\ \%$$

B2 Dipotassium phosphate (K_2HPO_4)

$$H_3PO_4 + 2KOH \rightarrow K_2HPO_4 + 2H_2O$$

	Reactants		Products	
	H_3PO_4	KOH	K_2HPO_4	H_2O
Molecular weight (g/mol)	98	56.1	174.2	18
Mass (g)	98	112.2	174.2	36

Mass allocation: Total mass = 174.2 + 36 = 210.2 g

$$K_2HPO_4 = \frac{174.2}{210.2} \times 100 = 82.9\ \%$$

$$H_2O = \frac{36}{210.2} \times 100 = 17.1\ \%$$

B3 Ammonium ferric citrate ($C_6H_{5+4y}Fe_xN_yO_7$)

$$Fe(OH)_2 + NH_3 + C_6H_8O_7 \rightarrow C_6H_9FeNO_7 + 2H_2O$$

	Reactants			Products	
	$Fe(OH)_2$	NH_3	$C_6H_8O_7$	$C_6H_9FeNO_7$	H_2O
Molecular weight (g/mol)	90	17	192	263	18
Mass (g)	90	17	192	263	36

Mass allocation: Total mass = 263 + 36 = 299 g

$$C_6H_9FeNO_7 = \frac{263}{299} \times 100 = 88\ \%$$

$$H_2O = \frac{36}{299} \times 100 = 12\ \%$$

B4 $EDTANa_2$ ($C_{10}H_{14}N_2O_8Na_2$)

$$EDTA + 2NaOH \rightarrow EDTANa_2 + 2H_2O$$

	Reactants		Products	
	EDTA	NaOH	$EDTANa_2$	H_2O
Molecular weight (g/mol)	292.24	40	336.21	18.015
Mass (g)	292.24	80	336.21	36.03

Mass allocation: Total mass = 336.21 + 36.03 = 372.24 g

$$EDTANa_2 = \frac{336.21}{372.24} \times 100 = 90.3\ \%$$

$$H_2O = \frac{36.03}{372.24} \times 100 = 9.7\ \%$$

B5 Manganese chloride ($MnCl_2$)

$$MnO_2 + 4HCl \rightarrow MnCl_2 + Cl_2 + H_2O$$

	Reactants		Products		
	MnO_2	HCl	$MnCl_2$	Cl_2	H_2O
Molecular weight (g/mol)	86.94	36.46	125.844	70.906	18.015
Mass (g)	86.94	145.84	125.844	70.906	36.03

Mass allocation: Total mass = 125.844 + 70.906 + 36.03 = 232.78 g

$$MnO_2 = \frac{125.844}{232.78} \times 100 = 54\ \%$$

$$Cl_2 = \frac{70.906}{232.78} \times 100 = 30.5\ \%$$

$$H_2O = \frac{36.03}{232.78} \times 100 = 15.5\ \%$$

B6 Sodium Molybdate (Na_2MoO_4)

$$MoO_3 + 2NaOH \rightarrow Na_2MoO_4 + H_2O$$

	Reactants		Products	
	MoO_3	NaOH	Na_2MoO_4	H_2O
Molecular weight (g/mol)	144	40	206	18
Mass (g)	144	80	206	18

Mass allocation: Total mass = 206 + 18 = 224 g

$$Na_2MoO_4 = \frac{206}{224} \times 100 = 92\ \%$$

$$H_2O = \frac{18}{224} \times 100 = 8\ \%$$

B7 Copper sulfate ($CuSO_4$)

$$CuO + H_2SO_4 \rightarrow CuSO_4 + H_2O$$

	Reactants		Products	
	CuO	**H_2SO_4**	**$CuSO_4$**	**H_2O**
Molecular weight (g/mol)	79.5	98	159.5	18
Mass (g)	79.5	98	159.5	18

Mass allocation: Total mass = 159.5 + 18 = 177.5 g

$$CuSO_4 = \frac{159.5}{177.5} \times 100 = 89.9\ \%$$

$$H_2O = \frac{18}{177.5} \times 100 = 10.1\ \%$$

B8 Cobalt (II) nitrate ($Co(NO_3)_2$)

$$CoO + 2HNO_3 \rightarrow Co(NO_3)_2 + H_2O$$

	Reactants		Products	
	CoO	**HNO_3**	**$Co(NO_3)_2$**	**H_2O**
Molecular weight (g/mol)	75	63	183	18
Mass (g)	75	126	183	18

Mass allocation: Total mass = 183 + 18 = 201 g

$$Co(NO_3)_2 = \frac{183}{201} \times 100 = 91\ \%$$

$$H_2O = \frac{18}{201} \times 100 = 9\ \%$$

Appendix C Calculation of Energy Requirement for Cultivation

C1 Energy Requirement for Lighting

Assumption: Light intensity is 5,000 lux (FAO, 1996).

Operating time is 24 hours per day.

Stage 1: 0.25 L (Erlenmeyer Flask)

A dimension of 250 milliliters Erlenmeyer flask is shown in Fig. B1.

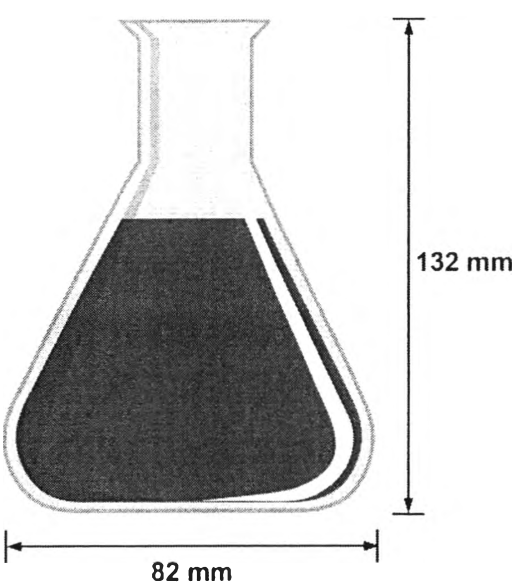

Figure C1 A 250 milliliters Erlenmeyer flask with dimension.

For calculation, the shape of Erlenmeyer flask is assumed to be a conical shape as shown in Fig. B2. Then Pythagorean theorem is applied to determine slant height (l) in order to determine illumination area further.

From Pythagorean theorem, $l = \sqrt{(132\ mm)^2 + (41\ mm)^2}$

$l = 138.221 \quad mm$

$l = 0.138 \quad m$

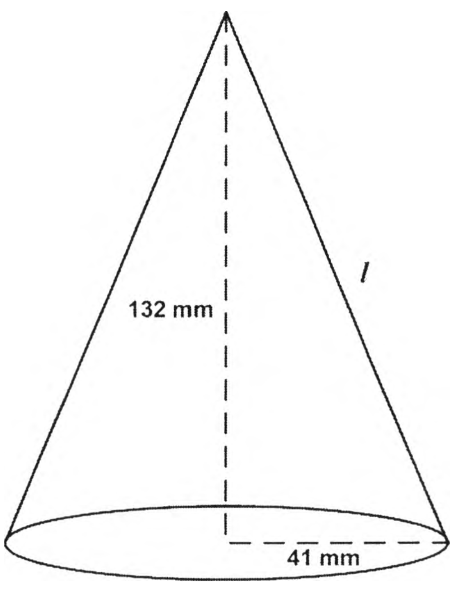

Figure C2 Cone with dimension.

Thus, $illumination\ area = \pi rl$

$$= \pi(0.041\ m)(0.138\ m)$$

$$= 0.018 \quad m^2$$

From light intensity, $luminous\ flux = (5{,}000\ lux)(0.018\ m^2)$

$$= 90 \quad lumen$$

$$= (90\ lumen)\left(\frac{1\ Watt}{683\ lumen}\right)$$

$$= 0.13 \quad Watt$$

Operate 24 hours for 3 days,

$$Energy = \left(\frac{0.13\ J}{s}\right)\left(\frac{3600\ s}{1\ hr}\right)\left(\frac{24\ hr}{1\ day}\right)(3\ days) = 33.696\ kJ$$

Stage 2: 2 L (Reagent Bottle)

A dimension of 2000 milliliters reagent bottle is shown in Fig. B3.

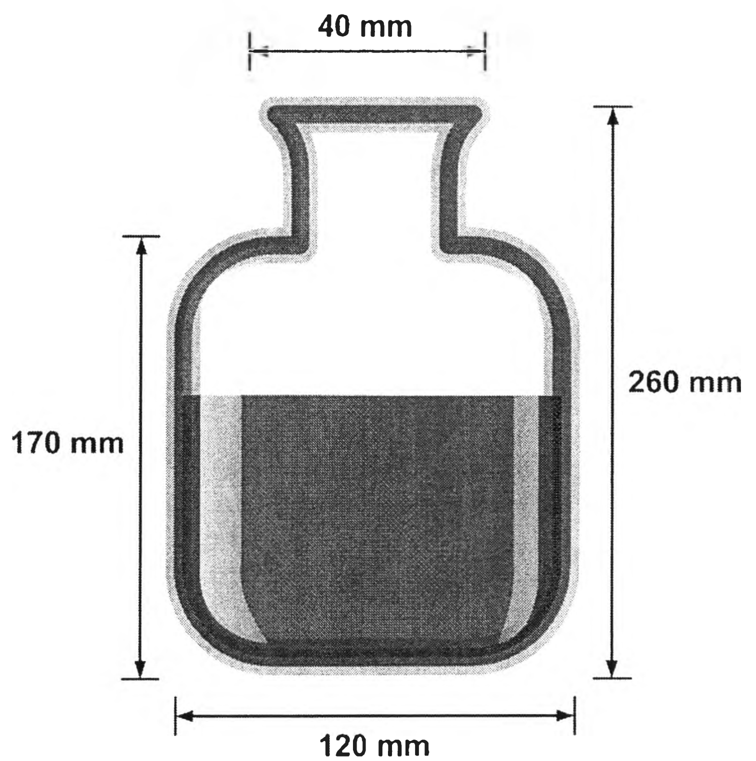

Figure C3 A 2000 milliliters reagent bottle with dimension.

For calculation, the shape of reagent bottle is assumed to be a cylindrical shape as shown in Fig. B4. Then illumination area which includes lateral area and top area is determined.

Thus, $illumination\ area = 2\pi rh + \pi r^2$

$$= 2\pi\left(\frac{0.12\ m}{2}\right)(0.26\ m) + \pi\left(\frac{0.12\ m}{2}\right)^2$$

$$= 0.109 \quad m^2$$

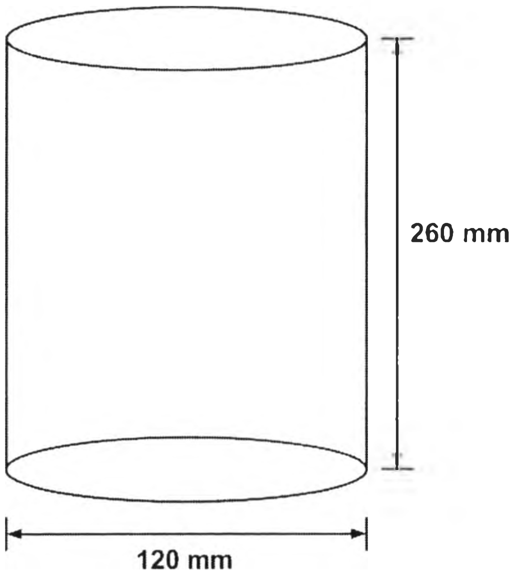

Figure C4 Cylinder with dimension.

From light intensity, $luminous\ flux = (5{,}000\ lux)(0.109\ m^2)$

$$= 545 \quad lumen$$

$$= (545\ lumen)\left(\frac{1\ Watt}{683\ lumen}\right)$$

$$= 0.8 \quad Watt$$

Operate 24 hours for 4 days,

$$Energy = \left(\frac{0.8\ J}{s}\right)\left(\frac{3600\ s}{1\ hr}\right)\left(\frac{24\ hr}{1\ day}\right)(4\ days) = 276.48\ kJ$$

Stage 3: 17 L (Rectangular Tank)

A dimension of 20 liters rectangular tank is shown in Fig. B5.

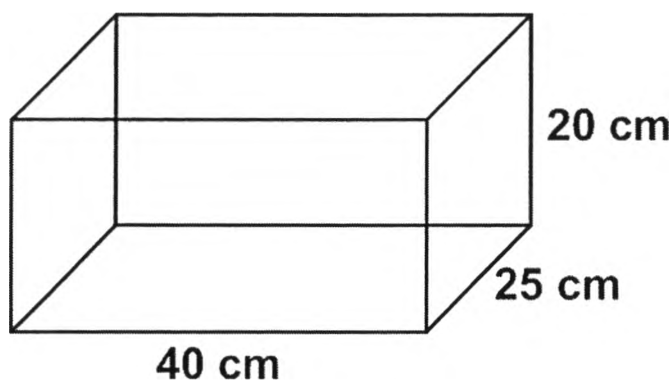

Figure C5 A 20 liters rectangular tank with dimension.

The illumination area which includes lateral area and top area is determined with the height of 17 cm.

$$illumination\ area = (0.4\ m)(0.17\ m) + 2(0.25\ m)(0.17\ m) + (0.4\ m)(0.25\ m)$$
$$= 0.136 + 0.085 + 0.1 \quad m^2$$
$$= 0.321 \quad m^2$$

From light intensity, $luminous\ flux = (5{,}000\ lux)(0.321\ m^2)$

$$= 1605 \quad lumen$$
$$= (1605\ lumen)\left(\frac{1\ Watt}{683\ lumen}\right)$$
$$= 2.35 \quad Watt$$

Operate 24 hours for 7 days,

$$Energy = \left(\frac{2.35\ J}{s}\right)\left(\frac{3600\ s}{1\ hr}\right)\left(\frac{24\ hr}{1\ day}\right)(7\ days) = 1421.28\ kJ$$

In summary, Table B1 shows the total energy required for lighting in the cultivation process.

Table C1 Energy requirement for lighting in cultivation process

Capacity (L)	**Energy requirement for lighting (kJ)**
0.25	33.696
2	276.48
17	1,421.28
Total	**1,732.456**

C2 Energy Requirement for Air Pumping

Basis: 1 second

Dissolved oxygen in freshwater at 25 °C and 760 mmHg is 8.244 mg/L (FAO, 1987).

Density of air at 25°C is 1.185 g/L.

Specification of air pump: Output = 150 L/min

Power = 100 W

Stage 1: 0.25 L

Oxygen

$$Dissolved\ oxygen = \left(\frac{8.244\ mg\ O_2}{L\ water}\right)(0.25\ L\ water) = 2.061\ mg\ O_2$$

Air

$$Air = (2.061\ mg\ O_2)\left(\frac{100\ mg\ air}{21\ mg\ O_2}\right) = 9.814\ mg\ air$$

Thus,

$$Energy = (9.814 \times 10^{-3} g\ air)\left(\frac{L\ air}{1.185\ g\ air}\right)\left(\frac{min}{150\ L\ air}\right)\left(\frac{100\ J}{s}\right)\left(\frac{60\ s}{1\ min}\right)$$

$$= 0.331\ J$$

Operate 24 hours for 3 days,

$$Energy = \left(\frac{0.331\ J}{s}\right)\left(\frac{3600\ s}{1\ hr}\right)\left(\frac{24\ hr}{1\ day}\right)(3\ days) = 0.086\ MJ$$

Stage 2: 2 L

Oxygen

$$Dissolved\ oxygen = \left(\frac{8.244\ mg\ O_2}{L\ water}\right)(2\ L\ water) = 16.488\ mg\ O_2$$

Air

$$Air = (16.488\ mg\ O_2)\left(\frac{100\ mg\ air}{21\ mg\ O_2}\right) = 78.514\ mg\ air$$

Thus,

$$Energy = (78.514 \times 10^{-3} g\ air)\left(\frac{L\ air}{1.185\ g\ air}\right)\left(\frac{min}{150\ L\ air}\right)\left(\frac{100\ J}{s}\right)\left(\frac{60\ s}{1\ min}\right)$$

$$= 2.65\ J$$

Operate 24 hours for 4 days,

$$Energy = \left(\frac{2.65\ J}{s}\right)\left(\frac{3600\ s}{1\ hr}\right)\left(\frac{24\ hr}{1\ day}\right)(4\ days) = 0.916\ MJ$$

Stage 3: 17 L

Oxygen

$$Dissolved\ oxygen = \left(\frac{8.244\ mg\ O_2}{L\ water}\right)(17\ L\ water) = 140.148\ mg\ O_2$$

Air

$$Air = (140.148\ mg\ O_2)\left(\frac{100\ mg\ air}{21\ mg\ O_2}\right) = 667.371\ mg\ air$$

Thus,

$$Energy = (667.371 \times 10^{-3} g\ air)\left(\frac{L\ air}{1.185\ g\ air}\right)\left(\frac{min}{150\ L\ air}\right)\left(\frac{100\ J}{s}\right)\left(\frac{60\ s}{1\ min}\right)$$

$$= 22.527\ J$$

Operate 24 hours for 7 days,

$$Energy = \left(\frac{22.527\ J}{s}\right)\left(\frac{3600\ s}{1\ hr}\right)\left(\frac{24\ hr}{1\ day}\right)(7\ days) = 13.624\ MJ$$

Stage 4: 100 L

Oxygen

$$Dissolved\ oxygen = \left(\frac{8.244\ mg\ O_2}{L\ water}\right)(100\ L\ water) = 824.4\ mg\ O_2$$

Air

$$Air = (824.4\ mg\ O_2)\left(\frac{100\ mg\ air}{21\ mg\ O_2}\right) = 3925.714\ mg\ air$$

Thus,

$$Energy = (3925.714 \times 10^{-3} g\ air)\left(\frac{L\ air}{1.185\ g\ air}\right)\left(\frac{min}{150\ L\ air}\right)\left(\frac{100\ J}{s}\right)\left(\frac{60\ s}{1\ min}\right)$$

$$= 132.514\ J$$

Operate 24 hours for 7 days,

$$Energy = \left(\frac{132.514\ J}{s}\right)\left(\frac{3600\ s}{1\ hr}\right)\left(\frac{24\ hr}{1\ day}\right)(7\ days) = 80.144\ MJ$$

In summary, Table B2 shows the total energy required for air pumping in the cultivation process.

Table C2 Energy requirement for air pumping in cultivation process

Capacity (L)	**Energy requirement for air pumping (MJ)**
0.25	0.086
2	0.916
17	13.624
100	80.144
Total	**94.77**

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1. Wibul, P.; Malakul, P.; Nithitanakul, M.; Pavasant, P.; Kangvansaichol, K.; and Papong, S. (2012, April 24) Life Cycle Assessment Study of Biofuel Production from Microalgae in Thailand: A Focus on Energy Efficiency and Global Warming Impact Reduction. Proceedings of the 3rd Research Symposium on Petrochemical and Materials Technology and the 18th PPC Symposium on Petroleum, Petrochemicals, and Polymers, Bangkok, Thailand.