International Baccalaureate Diploma Program

Extended Essay

Biology

HARVESTING ELECTRON TRANSPORT CHAIN ELECTRONS FROM CHLOROPLAST THYLAKOIDS OF *SPINACIA OLERACEA*

Research Question: To what extent can *Spinacia oleracea* chloroplast thylakoids electron transport chain electrons be harvested by an artificial electron carrier (DCPIP) and generate a voltage?

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INTRODUCTION

In light of the impending shortage of coal, gas and oil (Patzek & Croft, 2010) (Bentley, 2002) and global warming due to gases such as CO_2 (IPCC, 2007), some scientists searching for renewable and cleaner energy sources have been looking into the efficient biological process of photosynthesis (Fig. 1) for answers. Indeed, photosynthesis is "a complex molecular machinery [developed] for the efficient conversion of sunlight to chemical energy over the past 3 billion years, which to the present day has not been matched by any man-made technologies" (Kruse, Rupprecht, Mussgnug, Dismukes & Hankamer, 2005, p. 957).

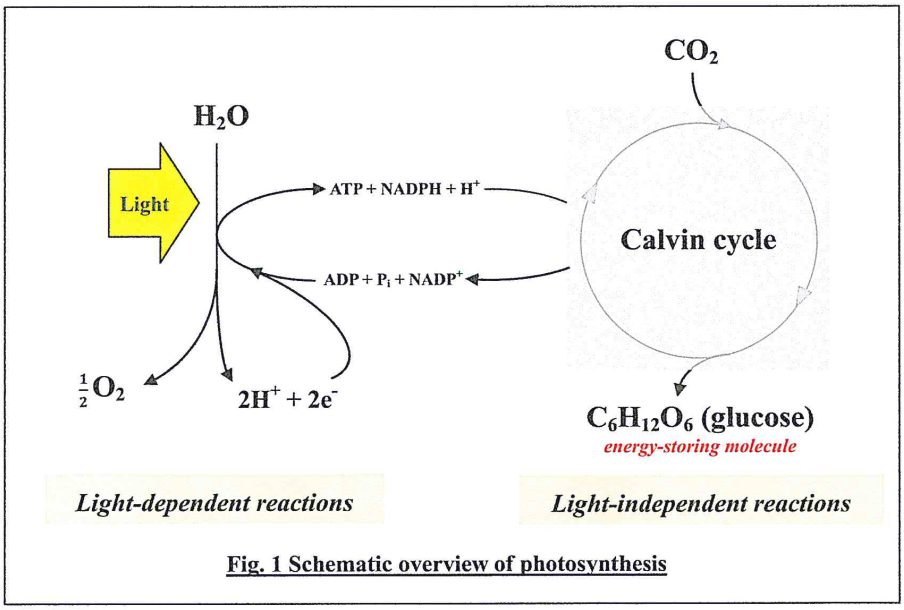

Fig. 1 Schematic overview of photosynthesis

The majority of current attempts to utilize photosynthesis-generated energy involves the usage of fixed carbon (Usui, 2010) and the production of bio-fuels e.g. diesel, ethanol

from photosynthetic crops or algae (Beal et al., 2012). Such approaches to utilize living systems would encounter a variety of problems. Energy is possibly lost through the organisms' miscellaneous metabolism necessary for survival and sustenance (Usui, 2010). Moreover, extraction and combustion of the bio-fuels are necessary for utilization, both of which could involve energy loss (Ky, 2011). Above all, the usage of crops for bio-fuel production as an alternate energy source has been claimed to worsen food shortage crisis, causing social outcry (Sexton, Rajagopal, Zilberman & Hochman, 2008).

The light-dependent reaction at the thylakoid membranes in chloroplasts, from which plant and algae photosynthesis starts, seems to offer an alternative. Due to insufficient available apparatus for studying algal chloroplast, this study focused on plants. At photosystem II (PSII), photo-induced charge separation (photolysis) is carried out by the

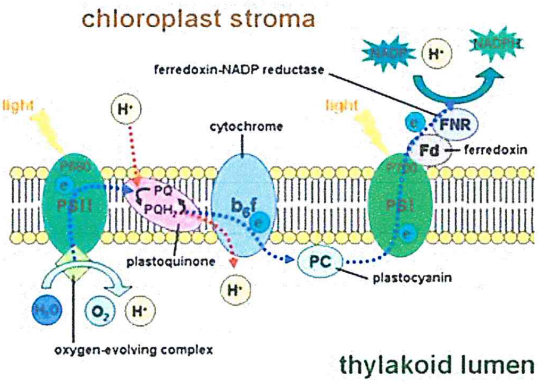

Fig. 2 Illustration of the electron transport chain (ETC) (cropped)
(Wikipedia, 2007)

oxygen-evolving complex (Renger, 2012) and chlorophyll after photo-activation. The protein complex splits water into oxygen, protons and electrons, among which the electrons are excited, shuttled through plastoquinone, cytochrome b_6f complex, plastocyanin and PSI, forming an electron transport chain (ETC) (Whitmarsh & Govinjee, n.d.) (Fig. 2).

Since these transfers at thylakoids are done with the help of respective electrochemical potentials of the ETC components, it is likely that certain external, foreign electron carriers (artificial electron carriers) could be used to divert the flow of electrons by redox reactions and bring electrons away from ETC, if this electron flow's mechanism is largely independent of its final destination. Thus, if the flow of electrons were successfully diverted, a voltage could potentially be generated by releasing the electron from the introduced artificial electron carrier. In short, electrical energy, instead of resultant chemical energy, can be obtained directly from photosynthesis via only light-dependent reactions; besides, synthesis of unnecessary bio-molecules at later stages of photosynthesis is avoided and, since the process can occur largely independently with other cellular components, only the thylakoid membranes are involved and needed in this approach. Most importantly, biological tissue and associated chemicals are not used for consume energy but for generating energy instead.

Carriers potentially with such ability include methylene blue, 2,6-dichlorophenol-indophenol (DCPIP), phenazine methosulfate, ferricyanide etc. Phenazine

methosulfate (Lynn, 1967) and ferricyanide (Drechsler & Neumann, 1982) have been found to inhibit chloroplast activity. Between methylene blue and DCPIP, I decided to use DCPIP for investigation as the thylakoid membrane-DCPIP couple electron transfer has been well documented (e.g. Park, Kelly, Drury & Sauer, 1966) as Hill Reaction and used to measure photosynthetic rate. DCPIP turns from blue to pink and eventually colorless as it is reduced to $DCPIPH_2$.

Since such an approach is a theoretically promising way to generate electrical energy from photosynthetic pathways, substantial prior study should be done to investigate the feasibility of the processes involved, especially the theoretical and biological aspects.

In this pilot study, spinach (*Spinacia oleracea*), a readily available dicotyledonous angiosperm, is chosen to be the source of thylakoid membranes as it has been commonly used for photosynthetic studies (e.g. Lilley, Fitzgerald, Rienits & Walker, 1975); data obtained can be easily compared with other studies and the study continued subsequently.

Ultimately, this study aims to answer the question: Can *Spinacia oleracea* chloroplast ETC electrons be harvested by an artificial electron carrier (DCPIP) and generate a voltage?

DESIGN AND METHODS

Corresponding to the central research question, the objective of this investigation is to evaluate *Spinacia oleracea* ETC electrons' ability to be harvested by DCPIP and to contribute to a voltage.

In order to answer the question, a specific set-up must be designed to enable *S. oleracea* ETC electrons to be produced, intercepted by DCPIP and, finally, accepted by a final electron acceptor after going through an external circuit where the voltage, if any, would be measured. Thus, the experiments would aim to investigate whether a voltage would be generated from such a specific set-up and condition, after successful harvesting of ETC electrons by DCPIP. Besides, it will be essential to ensure the voltage observed is the voltage from the biologically based process as explained by the central research question.

Hence, my first hypothesis is that the ETC electrons would be intercepted by DCPIP such that DCPIP would be reduced; acceptance of this hypothesis is a prerequisite before testing the second hypothesis. Next, with the null hypothesis, i.e. no voltage would be present, in mind, my second, main hypothesis is that a voltage would be generated; more specifically, a voltage around +0.55V (refer to “Part II: Reactions”).

Major parts of the set-up design are based on referenced materials (Giebel, n.d.) (Adams, n.d.) (Clark, n.d.) (Bennetto, 1990).

Thylakoid suspension

As mentioned above, *Spinacia oleracea* is chosen for its common usage in biological research. Since relevant reaction centers are located at thylakoid membranes, they must be isolated without other cellular components to avoid interference, while keeping the environment stable for reactions. However, it was impossible to exclude all other materials. Also, destruction of some thylakoid by enzymes, though minimized by ice-bath, was unavoidable to a certain extent. These were sources of error and could be prevented in future through a more profound isolation by multiple centrifugations in better-specialized media.

While some chloroplasts may have been isolated wholly, hypotonicity of reaction mixture would theoretically burst the outer membranes of chloroplast to expose the thylakoid membranes (Dean & Miskiewicz, 2003).

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Part I

DCPIP ETC-electron harvesting

Hill reaction was carried out to evaluate if electron interception by DCPIP occurs in light. In order to confirm that the decolorization of DCPIP was not due to e.g. thermo-decomposition due to lamp light, an oxidizing agent was added afterwards to see if DCPIP's color would be restored.

Overall design

The dependent variable was the color of solution. The independent variable was the presence of light, and subsequently the presence of oxidizing agent. The controlled variables are covered in Part II: Variables.

Part II

Container and membrane

The acrylic reaction-chamber container[1] has two ~10mL chambers, separated by a membrane sandwiched in the middle for proton exchange (Fig. 3). While a proton-exchange membrane would have been more effective, dialysis membrane was used due to availability issues.

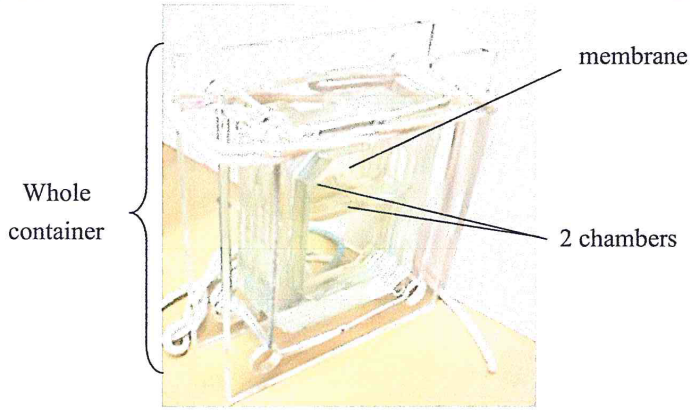

Fig. 3 Photo of reaction chamber container (with membrane and distilled water)

[1] Designed in CorelDraw and produced with laser-cutter; Appendix C

Final external electron acceptor

A final external electron acceptor was needed to react with electron-carrying (reduced) DCPIP for a voltage to be generated and measured. $Fe(NO_3)_{3(aq)}$ was chosen as Fe^{3+} is readily reduced, and $Fe(NO_3)_{3(aq)}$ does not very readily go through the dialysis membrane, which was confirmed beforehand by placing $Fe(NO_3)_{3(aq)}$ and water on either side of the membrane. No brown color of $Fe(NO_3)_{3(aq)}$ was observed on water's side after waiting for 5 minutes, which is beyond the time the experiments needed.

Reactions

The reactions involved (hereafter called "proposed pathway") are illustrated below.

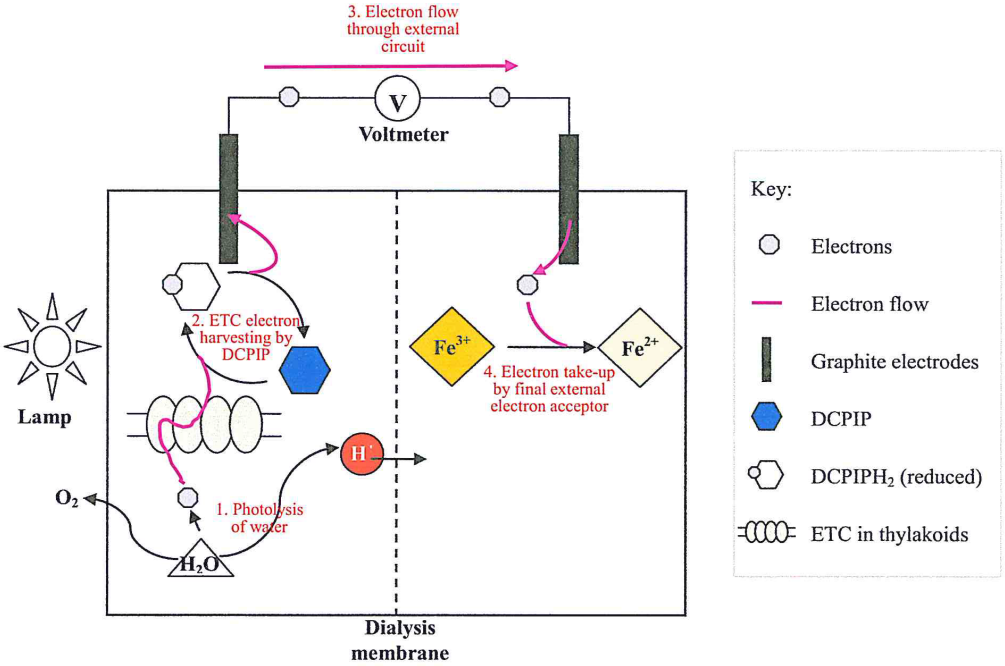

Fig. 4 Illustration of reactions and processes in the whole set-up

The DCPIP would intercept and harvest electrons, if possible, from plastoquinol (reduced plastoquinone) after PSII and before PSI (Dean & Miskiewicz, 2003), and become reduced as $DCPIPH_2$. DCPIP is blue while $DCPIPH_2$ is colorless.

Reactions that follow electron interception are as follows:

	Standard potential:	
Anode: $DCPIPH_{2\,(aq)} \rightarrow DCPIP_{\,(aq)} + 2H^+_{(aq)} + 2e^-$	-0.22V	(Melin & Lohmeier-Vogel, 2003)
Cathode: $Fe^{3+}_{(aq)} + e^- \rightarrow Fe^{2+}_{(aq)}$	+0.77V	(IBO, 2008)

Overall = +0.55V

Variables, Set-ups and trials

The measured dependent variable in the experiment is voltage. In order to ensure that the voltages measured were generated through the proposed pathway, a control (hereafter called "dark control") with identical composition but in darkness was set up for comparison. Hence, the independent variable was the presence of light. The main controlled variables include the container, volume and concentration of each solution, materials in each chamber, membrane, electrodes, temperature (in air-conditioned ambient environment) and plant used. The rationale of such design is that the control would account for any effects from the potential inherent chemical reactions of the chemical used, the chemicals' properties and the apparatus. Any voltage differences between the full and control set-ups would be due to the presence of light, which was assumed to only activate PSII to carry out photolysis in PSII, initiating the proposed pathway. Thus, the effect of electrons harvested from thylakoid ETC

would be the only probable reason for any voltage differences. It was assumed that the light would cause no significant effects to voltage other than through the proposed pathway.

The dark control was repeated thrice and full set-up seven times to increase reliability.

Apparatus and materials

Materials	Quantity
Distilled water	~2L
Ice	1 bucket-full
Spinacia oleracea leaves	5
Tris-sucrose buffer (0.3M sucrose, 0.2M Tris-HCl, 5mM $MgSO_4$)	~150mL
0.03 mM DCPIP solution	~150mL
0.1mM $K_2Cr_2O_{7(aq)}$	~30mL
Cheesecloth	6 pieces
1% $Fe(NO_3)_{3(aq)}$	~150mL
Dialysis membrane	1 piece
Aluminum foil	1 piece

Table 1 Table showing materials used

Apparatus	Quantity
Reaction chamber container	1
Scissors	1
15mL centrifuge tubes	3
Centrifuge	1
Test tubes	4
Filter funnel	1
10mL measuring cylinder	1
6mL syringes	3+
10mL syringes	3+
Lamp Details.	1
100mL beakers	~2
Mortar and pestle	1 set
Digital voltmeter	1
Graphite electrodes	2
Wires with clips	2
Container for ice bath	1
Fridge	1
Ruler	1

Table 2 Table showing apparatus used

Procedures

1. 5 deveined spinach leaves were cut with scissors into small pieces and grinded with pre-chilled mortar and pestle in 15mL ice-cold Tris-sucrose buffer into a paste.
2. The paste was filtered through 6 layers of cheesecloth via a filter funnel into a pre-chilled 15mL centrifuge tube.
3. After balancing, the tube was centrifuged at 3500 rpm for 5 minutes.
4. The supernatant was discarded and the dark-green pellet was re-suspended in 10mL ice-cold buffer.
5. The suspension was put with 10mL of additional ice-cold buffer into a test tube in an ice-bath.

Part I

6. With syringes, 9mL $DCPIP_{(aq)}$ and 1mL thylakoid suspension were added to a test tube.
7. Approximately half of the mixture was transferred to another test tube.
8. One test tube was wrapped in aluminum foil while the other was put in front of a lamp for 1min.
9. After observation, approximately half of the reaction mixture put under light was transferred to a new test tube.
10. About 5mL of $K_2Cr_2O_{7(aq)}$ and distilled water were added respectively to the two tubes. Color was observed.

Part II

11. A piece of dialysis membrane was cut to about 45 mm by 60 mm.
12. The reaction chamber was assembled with the membrane in between. One chamber was labeled 'A' and the other 'B'.
13. With syringes, 5mL of $Fe(NO_3)_{3(aq)}$ was added to chamber A and 4.5mL of DCPIP and 0.5mL of thylakoid suspension were added to chamber B. The container was shaken lightly to mix the solutions.
14. A lamp was placed 10cm away, facing chamber B.
15. Graphite electrodes were washed with distilled water thoroughly and the voltmeter was re-calibrated if necessary.
16. Graphite electrodes were inserted into the two chambers and connected with wires to the digital voltmeter.
17. The voltage was recorded after waiting for 30 seconds, giving photosynthetic components time to adapt and activate.
18. Steps 13-17 were repeated seven times, after clearing, washing and rinsing the container with distilled water every time.
19. Steps 13, 15-18 were repeated with aluminum foil wrapped around the container. The set-up was repeated thrice only in step 18.

Additional notes and Precautions

- Steps 1-5 were done swiftly and in low temperatures whenever possible to minimize miscellaneous biochemical reactions including thylakoid degradation by enzymes released from cell debris (Clegg, 2007, p.402).
- Step 19 was carried out with the laboratory light dimmed to minimize ambient light.
- Gloves, laboratory coat and safety goggles were worn as the chemicals are mildly harmful.

Statistical significance testing

Voltage data were treated with the non-parametric Mann-Whitney U test to test for significance of results because n was too small to assume normal distribution for unpaired t-test.

RESULTS AND ANALYSIS

Observations, data and representation

Notable qualitative observations:

- DCPIP was decolorized when mixed with thylakoid suspension under light. No decolorization was observed in darkness.

  The decolorized reaction mixture was re-colorized as $K_2Cr_2O_{7(aq)}$ was added (Fig. 5)

- In Part II, dark green congregations of below $1mm^3$ were found in most set-ups after experiment in the thylakoid suspension and DCPIP mixture.

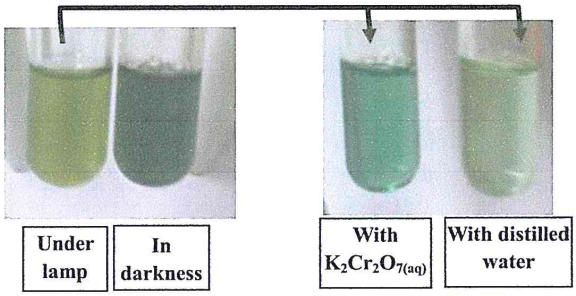

Fig. 5 Photos from Part I experiment

Set-up	Voltage (V) (±0.01)								n	Mean (V)	Median (V)	(unbiased) Standard deviation (V)
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8				
Dark control	0.06	0.05	0.04	0.04	/	/	/	/	4	0.0475	0.0450	0.0096
Full set-up	0.08	0.07	0.06	0.07	0.10	0.09	0.08	0.08	8	0.0788	0.0800	0.0125

Table 3 Table showing data collected and primary analysis

Mann-Whitney U test statistic *U*	0.5
U critical value at α=0.05	4
U critical value at α=0.01	1

Table 4 Table showing results of Mann-Whitney U Test[2] and Mann-Whitney U critical values[3]

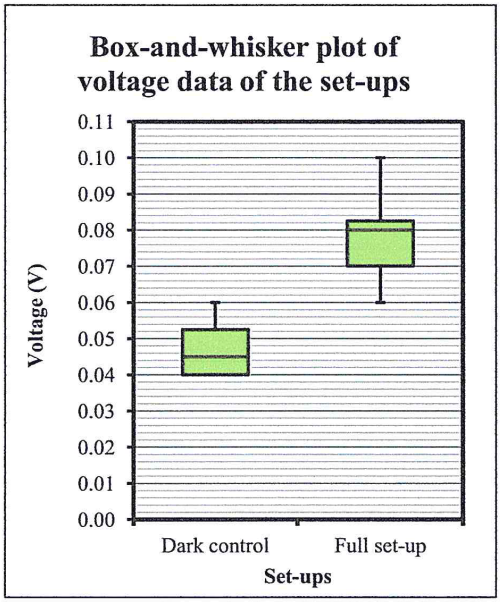

Fig. 6 Box-and-whisker plot of voltage data of the set-ups

explain this, as it is not often used. Show understanding.

[2] Appendix A
[3] Appendix B

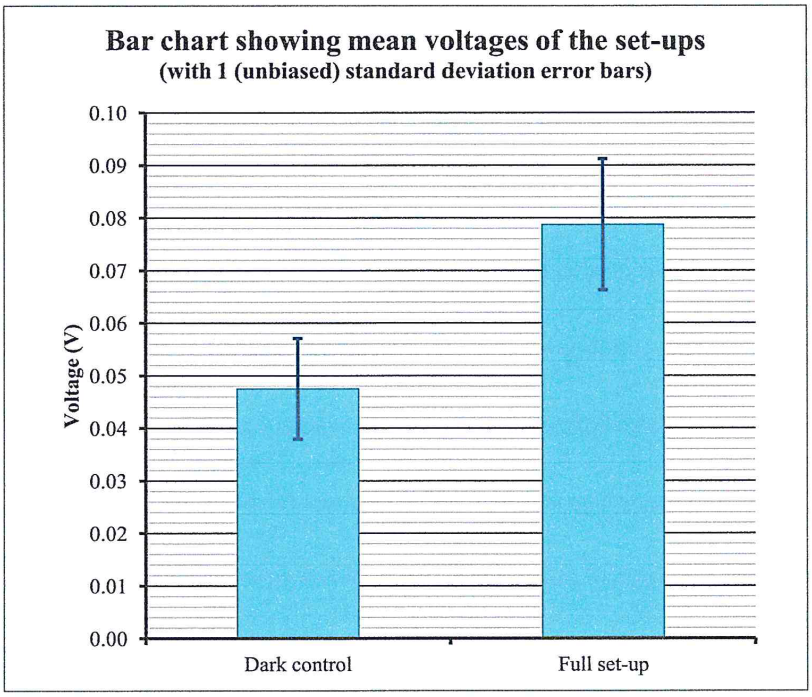

Fig. 7 Bar chart showing mean voltages of the set-ups

From the results of Part I, since the electrochemical reversible decolorization of DCPIP was observed with thylakoids under light only, DCPIP was shown to be reduced by a light-driven electrochemical reaction.

From the experiments carried out in Part II, voltage values were recorded from 4 trials of dark control and 8 trials of full set-up. No outlying data points were observed, so no data points needed to be discarded. All voltage readings were positive, but were under or equal to a mere 0.10V.

Voltage variation and averages

The voltage readings of dark control range from 0.04V to 0.06V, while those of full set-up range from 0.06V to 0.10V. As illustrated in Table 3 and Fig. 6, the medians of the two sets of voltage readings differ by 0.035V, or 0.04±0.02V if with regard to the uncertainty due to voltmeter used. It is clear in the box-and-whisker plot between the two sets of data (Fig. 6) that the 25th quartile of full-set-up is higher than the 75th quartile of dark control by almost 0.02V, indicating a large difference between the two sets of voltage readings.

Similar to the median and quartiles serving as a reference, the mean and standard deviation also give positive conclusions. The mean full set-up voltage is higher than the mean of dark control voltage by 0.0313V, or 0.03±0.02V when accounting for voltmeter uncertainty. It is clear in Fig. 7 that the respective means of two sets of data differ by a considerable extent. Considering a deviation of 1σ, the voltage of full set-up is still greater than that of dark control because the lower end of full set-up's error bar is well above the upper end of dark control's error bar.

Mann-Whitney U test

In order to establish the significance of the data obtained more concretely, the Mann-Whitney U test statistic was used. As outlined in Table 4, the U obtained was 0.5. Setting significance level at 0.05, as the U obtained, 0.5, is smaller than the critical value, 4,

the result is deemed significant. To evaluate further an even greater significance of data, the set significance level was subsequently changed to 0.01. The U obtained is still smaller than the critical value, 1, which implies that the difference obtained between the two set-ups is significant with >99% confidence. Thus, the null hypothesis that no voltage would be generated from such a specific set-up is rejected, as the specific set-up shows a very significantly different voltage from the background voltage shown by the dark control. Moreover, since the data shows that full set-up has higher voltage than the dark control, coupled with the rejection of null hypothesis, it is indicated that the specific set-up designed for the proposed pathway has contributed to significant voltage, such that the voltage reading became higher; in other words, at least a part of the second hypothesis can be accepted. Specifically, however, the voltage did not reach 0.55V as hypothesized.

DISCUSSION AND EVALUATION

Photolysis, ETC electron harvesting, Voltage generation

First of all, the results of Part I is concrete support for the presence of DCPIP reduction due to light reactions in the thylakoid membranes. Since no other causes of light-dependent reduction are known, it is with confidence that the first prerequisite hypothesis is approved, that electrons from the ETC were indeed intercepted by (i.e. transferred to) DCPIP.

Moreover, using the analysis shown above we can conclude with confidence that under light the combination of thylakoid suspension and chemicals in the specific set-up gave rise to about 0.03 to 0.04V greater voltage than when in darkness. However, as noted before, it is essential to ensure that the voltage reflects the reaction by the proposed pathway via the thylakoid electron harvesting, as explained to be the central studied subject of investigation. Since photolysis requires the presence of light to be carried out i.e. should not have been able to be carried out in the dark control, the voltage increase observed in the full set-up should, at least partially, be due to photolysis, especially with support from Part I that photolysis and DCPIP-harvesting did occur in such conditions. More specifically, the voltage should be generated from the release of thylakoid ETC electrons DCPIP harvested through the external circuit to the iron (III) ions on the other side of set-up, which requires the occurrence of photolysis.

Possible misestimation of voltage

However, it is possible that the voltage increase was caused by reasons other than the provision of DCPIP-harvested electrons from the ETC. Firstly, the light might have driven unknown miscellaneous reactions in the substance mixture of the whole set-up, especially since the thylakoid suspension contains a mixture of various chemicals from buffer and cell debris. Secondly, some heat could have been transferred from the lamp to the reaction mixture. In other words, the temperature may not have been controlled as desired, which could have caused a change in background voltage, such as unforeseen temperature-driven reactions in the substance mixture. Modifications to the set-up should be done in the future to tackle this issue, perhaps by including a separate water chamber exterior of the reaction chambers. Thirdly, some electrons could have been transferred directly from the thylakoid ETC to the graphite electrode instead of through the harvesting action of DCPIP, which is possible if some thylakoids were attached to the electrode directly. Nonetheless, most of these possible reasons of overestimation are unlikely and account for a negligible voltage difference.

On the other hand, the voltage difference measured might not represent all the voltage DCPIP-harvested photolysis-generated electrons were able to generate. Firstly, the aluminum foil did not cover the entirety of the reaction chamber due to the protruding electrodes. Some light might have been present, i.e. complete darkness might not have been achieved.

Therefore, some photolysis might have carried out in the dark control, causing a portion of the voltage generated by the proposed pathway to be excluded from the arrived value when subtracting voltage value of dark control from that of full set-up. Secondly, setting up the reaction chamber required time under ambient light, although precautions were taken to minimize the effect. Therefore, in the time prior to light insulation by aluminum foil, the photosynthetic components e.g. PSII might have already started to carry out reactions. Therefore, some of the voltage measured in the dark control set-ups could have been due to the electrons already generated by photolysis, harvested by DCPIP and stored in $DCPIPH_2$ during the preparation of set-up.

Hence, it is both possible that the voltage difference recorded is an overestimation or an underestimation of the actual voltage generated through the proposed pathway via the photosynthetic reactions. Nevertheless, the potential reasons are mostly negligible in their effects and likely cancelled out. By comparison with the confident and significant voltage increase due to presence of light, we can be confident to conclude the voltage increase recorded is a fair reflection of voltage from electrons in thylakoid ETC.

Background voltage in dark control

Apart from mentioned reasons, there are other probable reasons for the unexpected inherent voltage in the dark control. First, $Fe(NO_3)_3$ has a low pH; as pH difference is closely

related to electric potential (Starck, 2000), the relatively low pH of $Fe(NO_3)_3$ is likely to have been a contributing factor to the inherent voltage measured in the dark control. Moreover, cell debris, which were inevitably present in the thylakoid suspension, usually have a negative charge (Yavorsky, Blanck, Lambalot & Brunkow, 2003), and this would further enlarge the pH difference between the two reaction chambers and contribute to an unforeseen inherent voltage.

Voltage generation efficiency

If the resultant voltage reflects wholly the effect of intercepted electrons from *S. oleracea* thylakoid ETCs, the voltage the proposed pathway contributed will be 0.03±0.02V, which is only 5.45% of the theoretical 0.55V voltage. The unexpectedly low voltage generation raises doubt not only on the practicality of using the proposed pathway to generate energy but also on the presence of actual contribution by the proposed pathway in the generation of the extra voltage. Fortunately, a number of probable reasons have been noted apart from the mentioned issues with thylakoid suspension impurities, cellular structure destruction by enzymes and choice of membrane. As observed, dark green congregations were observed in the DCPIP-thylakoid suspension mixture after certain duration of reaction. These congregations were likely to be thylakoids or chloroplasts. While the reason for the formation of such congregations is unknown, this would most likely have caused a reduction

in effective photolysis and electron harvesting by DCPIP due to the fact that fewer ETCs were exposed to solution. It is also likely that a transformation of the molecular biological characteristics of associated membranes and proteins was involved in the congregation formation, which would further undermine the efficiency of the proposed pathway. In addition, since PSII is a protein complex, it is naturally highly sensitive to the medium conditions, such as pH and temperature. Due to the possible heat transfer from the lamp and the low pH of $Fe(NO_3)_{3(aq)}$ (despite the membrane separation and buffer used), it was possible that the conditions were outside the optimal range of activity of the ETC components involved. If so, the photosynthetic components involved in the proposed pathway would have been in a condition of a low efficiency and the results would not have reflected the voltage that could be generated when they are in the optimal condition. Lastly, the concentrations of solutions used were very low, which is also a probable factor. Ultimately, the voltage increase caused by the proposed pathway might have been largely limited by other factors, which would explain the low voltage generation recorded. Therefore, the results still form a strong support for the proposed pathway's contribution in voltage generation.

CONCLUSION

The experiments carried out firstly established that *S. oleracea* chloroplast ETC electrons could be harvested by DCPIP. Secondly, a specially designed set-up, compared with a dark control, showed that the harvested electrons could contribute to a voltage generation if released to a final electron acceptor.

The results confirmed the *S. oleracea* ETC electron harvesting by DCPIP, as expected by Hill Reaction. The voltage of full set-up was greater than the voltage of dark control by, in mean, 0.03±0.02V or, in median, 0.04±0.02V (compared to 0.55V in theory) with >99% confidence. Although a number of unpredicted factors were noted, the voltage value found can still be regarded as the approximate voltage actually generated by the DCPIP-harvested electrons, as the reasons are largely negligible.

Consequently, the first hypothesis is accepted and the second only partially accepted, as the voltage did not reach the level stated in the second half of the second hypothesis. Nonetheless, some factors that were identified suggest that the voltage could be nearer the predicted value if in more ideal conditions. All in all, it was found that *S. oleracea* chloroplast thylakoids electron transport chain electrons can indeed be harvested by an artificial electron carrier DCPIP and, moreover, generate a voltage.

Apart from the mentioned ones above, other surprising observations were also made, including the congregation of green tissues from the thylakoid suspension after some time. This could be further studied upon both in or out of context of the proposed pathway.

It is important to note how the results of this investigation is very promising in its demonstration of the ability of plant chloroplast ETC electrons to be diverted from the normal flow and released in an external environment; the reactions carried out by plant chloroplasts are flexible enough for their properties to accommodate such manipulations. However, more substantial studies must be made before any value such proposed pathway could be proved to have in molecular biology and the search for alternate energy sources in resource and climate crises. Further investigation should strive to give a more comprehensive picture by investigating the differences in the studied property of other species, including algal ones, since it is inappropriate to generalize the property of ETC electrons from the study on a single plant species. The sustainability of the pathway plus energy investment in growing chloroplasts must be investigated, by, e.g., manipulating duration and reactants and evaluating cultivation efficiency. Set-ups with other dimensions, such as larger scale for industrial-scale studies or smaller scale for nano-scale investigations, could be considered. Chemicals alternative to those used in this investigation could also be used to find better combinations that realize the potential of ETC voltage harvesting, especially in light of the pH issue. Lastly, more trials with more accurate apparatus should be done to increase both the reliability and accuracy of data: the large uncertainty value caused by the voltmeter in this investigation exemplifies apparatus-related issues; the number of trials in this study was highly limited due to limited resource available.

REFERENCES

Adams, M. (n.d.). *The Hill Reaction in Isolated Chloroplasts.* Retrieved April 22, 2013, from http://www.easternct.edu/~adams/Resources/Lab4%20Hill%20Rx.pdf

Beal, C.M. et al. (2012). Comprehensive Evaluation of Algal Biofuel Production: Experimental and Target Results. *Energies*, 5, 1943-1981.

Bennetto, H.P. (1990). Electricity generation by microorganisms. *Biotechnology Education*, 1(4), 163-168.

Bentley, R.W. (2002) Global oil & gas depletion: an overview. *Energy Policy*. 30, 189-205.

Clark, J. (n.d.). *The Hill Reaction in Chloroplasts.* Retrieved April 22, 2013, from http://faculty.weber.edu/jclark1/Cell%20Biology%20Labs/Hill%20Reaction.pdf

Clegg, C.J. (2007). *Biology for the IB Diploma* (p.402). London: Hodder Education.

Dean, R.L., & Miskiewicz, E. (2003). Rates of Electron Transport in the Thylakoid Membranes of Isolated, Illuminated Chloroplasts Are Enhanced in the Presence of Ammonium Chloride. *Biochemistry and Molecular Biology Education,* 31(6), 410-417.

Drechsler, Z., & Neumann, J. (1982). Inhibition of Oxygen Evolution in Chloroplasts by Ferricyanide. *Plant Physiology,* 70, 840-843.

Giebel, P.E. (n.d.). *Extraction of Chloroplasts from Plant Tissue and Their Use in Demonstrating the Hill Reaction.* Retrieved April 22, 2013, from http://www.ableweb.org/volumes/vol-2/3-giebel.pdf

Hole, G. (n.d.). *The Mann-Whitney test.* Retrieved September 17, 2013, from http://www.sussex.ac.uk/Users/grahamh/RM1web/MannWhitneyHandout%202011.pdf

Intergovernmental Panel on Climate Change. (2007). *Climate Change 2007: Synthesis Report.* Retrieved August 26, 2013, from http://www.ipcc.ch/pdf/assessment-report/ar4/syr/ar4_syr.pdf

International Baccalaureate Organization. (2008). *Diploma Programme: Chemistry data booklet.* Chippenham, Wiltshire: Antony Rowe Ltd.

Kruse, O., Rupprecht, J., Mussgnug, J.H., Dismukes, G.C., & Hankamer, B. (2005). Photosynthesis: a blueprint for solar energy capture and biohydrogen production technologies. *Photochemical & Photobiological Sciences,* 4, 957-969.

Ky, J. (2011, November 18). Algal Biodiesel as an Energy Source?. Retrieved August 27, 2013, from http://www.yalescientific.org/2011/11/algal-biodiesel-as-an-energy-source/

Laverty, W.H. (n.d.). *Critical Values for the Wilcoxon/Mann-Whitney Test (U).* Retrieved September 17, 2013, from http://math.usask.ca/~laverty/S245/Tables/wmw.pdf

Lilley, R.M., Fitzgerald, M.P., Rienits, K.G., & Walker, D.A. (1975). Criteria of Intactness and the Photosynthetic Activity of Spinach Chloroplast Preparations. *New Phytologist,* 75, 1-10.

Lynn, W.S. (1967). Inhibition of Photophosphorylation by Phenazine Methosulfate. *The Journal of Biological Chemistry,* 242, 2186-2191.

Melin, A.D., & Lohmeier-Vogel, E.M. (2003). Using Cytochome c to Monitor Electron Transport and Inhibition in Beef Heart Submitochondrial Particles. *Biochemistry and Molecular Biology Education,* 32(1), 39-44.

Park, R.B., Kelly, J., Drury, S., & Sauer, K. (1966). The Hill Reaction of Chloroplasts Isolated From Glutaraldehyde-Fixed Spinach Leaves. *Botany,* 55, 1056-1062.

Patzek T.W., & Croft, G.D. (2010). A global coal production forecast with multi-Hubbert cycle analysis. *Energy*, 35, 3109-3122

Renger, G. (2012). Mechanism of light induced water splitting in Photosystem II of oxygen evolving photosynthetic organisms. *Biochimica et Biophysica Acta,* 1817, 1164-1176.

Sexton, S., Rajagopal, D., Zilberman, D, & Hochman, G. (2008). Food Versus Fuel: How Biofuels Make Food More Costly and Gasoline Cheaper. *Agricultural and Resource Economics Update*, 12(1), 1-6.

Starck, J. (2000). *pH measurement*. Retrieved September 17, 2013, from http://www.allaboutcircuits.com/vol_1/chpt_9/6.html

Usui, T. (2010, November 28). *Direct Harvesting of Electricity from Plant Photosynthesis.* Retrieved August 26, 2013, from http://large.stanford.edu/courses/2010/ph240/usui2/

Whitmarsh, J., & Govinjee. (n.d.). *The Photosynthetic Process.* Retrieved September 18, 2013, from http://www.life.illinois.edu/govindjee/paper/gov.html

Wikipedia. (2007). *Thylakoid membrane* [illustration]. Retrieved August 25, 2013, from http://en.wikipedia.org/wiki/File:Thylakoid_membrane.png

Yavorsky, D., Blanck, R., Lambalot, C., & Brunkow, R. (2003, March 2). The Clarification of Bioreactor Cell Cultures for Biopharmaceuticals. *Pharmaceutical Technology,* 27(3), 62-77.

APPENDICES

Appendix A: Calculation of U for Mann-Whitney U test

Set-up	Dark control	Full set-up
Rank of data	4.5	9
	3	6.5
	1.5	4.5
	1.5	6.5
	/	12
	/	11
	/	9
	/	9
Sum of ranks	10.5	67.5

Table showing ranking of data

$$U = n_1 \times n_2 + n_L \times (n_L+1) \div 2 - \sum r_L \qquad \text{(Hole, n.d.)}$$

$$= 4 \times 8 + 8 \times (8+1) \div 2 - 67.5$$

$$= 0.5$$

Appendix B: U critical value table (cropped) (Laverty, n.d.)

Critical Values for the Wilcoxon/Mann-Whitney Test (U)

Nondirectional α=.05 (Directional α=.025)

n_1 \ n_2	1	2	3	4	5	6	7	8	9	10
1	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	0	0	0
3	-	-	-	-	0	1	1	2	2	3
4	-	-	-	0	1	2	3	4	4	5
5	-	-	0	1	2	3	5	6	7	8
6	-	-	1	2	3	5	6	8	10	11
7	-	-	1	3	5	6	8	10	12	14
8	-	0	2	4	6	8	10	13	15	17

Nondirectional α=.01 (Directional α=.005)

n_1 \ n_2	1	2	3	4	5	6	7	8	9	10
1	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	0	0
4	-	-	-			0	0	1	1	2
5	-	-	-		0	1	1	2	3	4
6	-	-	-	0	1	2	3	4	5	6
7	-	-	-	0	1	3	4	6	7	9
8	-	-	-	1	2	4	6	7	9	11

Appendix C: Reaction chamber container design for laser cutter (for production)