Project Report - Inspo Research and Innovation Competition

Applying Iodine Photochemistry: Investigation into the Photochemical Reaction of Ammonium-Iodine-Oxalate as a Novel Method of CO_2 Reactant-Supplementation for Photosynthetic Processes as Observed in *Phaseolus vulgaris*

Sean Ticsay†, Eunice Fabricante†

†Author; Grade 11 student; Sisler High School, Winnipeg, Manitoba, Canada

Received: 2020.06.20

Summary

Given past experimentation, the development of a novel method to accelerate photosynthesis by the photo-redox catalysis induced supplementation of CO2 gas from the endergonic reaction of Ammonium-Iodine-Oxalate, as observed with *Phaseolus vulgaris*.

Introduction

In previous theoretical chemistry experiments performed by the authors, it was determined that LED-light best catalyzed the photochemical reaction of ammonium-iodine-oxalate and that said reaction plentifully yielded CO2. Given that reaction rate and CO2 production are directly proportional, further speculation introduced the question: Does changing the LED-light wavelength possess considerable effects in CO2 production by plausibly accelerating the reaction rate? Applying the fact that CO2 is imperative in photosynthesis, a conclusive outcome can be determined by analyzing plant growth. This project was performed in attempt to answer the very question.

Theory

The above stated reaction can be more specifically described as an aqueous solution of ammonium-iodine-oxalate, of which is initially black in colour, undergoing a photochemical kinetic endergonic reduction-oxidation reaction, wherein iodine is reduced to iodide and oxalate oxidized into carbon dioxide (the ammonium is unchanged as it is a buffer). The reaction produces a new aqueous solution now of ammonium-iodide-carbon dioxide, of which is now white in colour. This reaction is modelled using the following chemical equation:

$$2(NH_4)^{1+} + C_2O_4^{2-} + I_2 \Rightarrow 2(NH_4)^{1+} + 2CO_2 + 2I^{1-}$$

Background Research

Preliminary research and enquiries were done using relevant sources and are as follows: chemical safety and correctness of the chemical equation via the University of Manitoba Department of Chemistry; iodine and CO2 effects (postulation that iodine promotes plant biofortification and evidence showing that rate of photosynthesis increases in CO2-elevated environments [1][2]) via the National Center for Biotechnology Information; and exploring and applying analytical chemistry methods via the University of Toronto Department of Chemistry.

Hypothesis

If we test to determine the change in carbon dioxide yield, and consequently the rate of plant growth, in the process of photochemically reacting an ammonium-iodine-oxalate solution, then we will say that the plants exposed to monochromatic violet light will have a more developed growth compared to the other plants under different wavelengths.

The reasoning is that violet-light has the shortest wavelength of visible light (415nm) thus it contains the highest amount of quanta energy (2.988 eV or 1.6022×10^{-19} J) compared to the rest of the visible light (VL) wavelengths. This is known for wavelength and energy are inversely proportional according to a derived version of Planck's Equation ($E = hc/\lambda$). If the solution is exposed to more energy, catalysis of the reduction-oxidation reaction is improved, provided that photon energy is the catalytic force of the reaction.

VARIABLES

Independent & Dependent Variables

The wavelength of monochromatic light that plant samples are exposed to, ranging from 415nm to 685nm, would be the variable that is changed. The influenced variable would be the CO_2 yield and consequent rate of plant growth (see 'Data Collection Methodology').

Key Controlled Variables

The following were controlled throughout the experiment: the amount of solution per sample (accurately measured 10mL & replaced weekly), the amount of water given per sample (10mL ±0.25mL & watered every-other-day), the length of light exposure time (12 hours daily on the mark), the lights were monochromatic in each of their respective colours, and there were control samples (that included No-Solution, No-Light, White-Light/No-Filter, Sunlight, & Exhibit-Samples).

METHODOLOGIES AND PROCEDURE

Data Collection Methodology

Data points were collected using five methods deemed most accurate by the authors:
1) Final Biomass/Dry Weight – comparison of gram mass of all final dried plant matter;
2) System Weight – daily comparison of the weight of each plant growth-pod with respect to pod weight from the previous day and weight of the other pods;
3) Plant Height – daily millimetre comparisons of plant height using flexible Tailor's Tape;
4) Soil pH – measuring growth effects due to the known alkalinity of the solution; and
5) Soil Temperature – measuring to see if the solution caused change in centigrade temperature as plants have preferred temperatures to grow in.

Statistical Methodology

The final data was compiled using the mean averages from each respective data collection method. The average used for pH was mean because of the fact that soil pH followed an alkaline to neutral cycle beginning at the replacement of the solutions; using median and mode would be inaccurate. To add, temperature was maintained at an average range of 19-20 Celsius therefore mean-average was the more accurate methodology.

The experiment was conducted twice to reduce error. Two 40 day trials were performed allowing sufficient time for plant growth (it was observed that germination began within or around the first week - earlier than anticipated, therefore 40 days was decided as an ample amount of time). The two trials, using a total of 80 independent samples, amassed 6240 data points.

Materials

The necessary materials used in the project, in no particular order, include: plastic cups (large and small sizes), potting soil, black bean seeds, tape (Scotch, duct, & masking), distilled water, household ammonia, iodine (sold as Tincture of Iodine), oxalic acid (sold as Bar Keepers Friend®), transfer pipettes, a 100mL beaker, a 10mL graduated cylinder, a stirring rod, a digital scale, a thermometer, pH paper, Tailor's Tape, aluminum foil, rubber gloves, safety goggles, a flashlight, boxes, colour filters (ROYGBV monochromatic), black paint and painting utilities, Christmas lights (preferably white or clear), binder clips, elastic-bands, a permanent marker, a notebook & writing utensils, and a baking pan.

Apparatuses

Three unique apparatuses were constructed that each served a beneficial purpose towards the project in regards to accuracy and control:

1) Growth Pods – research showed that the amounts of chemical components used for the solution would be toxic to the plants. Therefore, special containers were constructed where a smaller cup would be suspended over a larger cup and they together would be covered by another larger cup (see Diag. 1). The plant and soil would go in the small cup while the solution in the bottom larger cup. This allowed a separation between plant and solution. The solution would produce CO2 that would rise but not escape due to the larger cup cover, thus getting absorbed by the plants. The authors note that this resembled a somewhat "Double-Boiler Contraption";

DIAGRAM 1

2) Light Boxes – these are boxes constructed to house growth pods under specific luminous conditions. The interior was divided into four quadrants with each covered in aluminum foil (except for the No-Light control quadrant which was painted black). Each quadrant had a hole on the roof where the filters and light would be placed to stimulate a monochromatic environment. The exterior was wrapped in duct tape to prevent light from escaping and all possible gaps in the opening flaps were sealed/taped tightly when closed;

3) Filtered-Flashlight-Device (FFD) – a problem arose when it was realized that light would be required in order to collect observational data. To combat the issue that the light needed to be of the same respective wavelength per unique sample, an apparatus, wherein a flashlight was suspended above the work table and its light-hole was covered by a coloured filter, was constructed. The filter would simply be replaced with the proper colour whenever required. This prevented light-contamination during observations.

Concocting the Ammonium-Iodine-Oxalate Solution

The solution was concocted by first combining 7.5g of oxalic acid and 75mL of distilled water into the 100mL beaker. The two were stirred and then 75mL of ammonia was added when the crystals dissolved. This new solution of ammonium-oxalate was again stirred and then 9mL of iodine added. The reaction began immediately as the solution turns black.

Jotting Methodology

To reduce the amount of time that the pods were outside the light boxes, all collected values and observations were swiftly jotted and then organized neatly only after all data collection was complete and the pods returned. This methodology utilized shorthand notation.

Collecting Empirical Data

All empirical data collections were done under FFD (with the exception of pH determination). Furthermore, all empirical data collections were done before the plants were watered. Soil temperature was collected by placing a glass stick thermometer within the soil for 20 seconds. System weight was collected by placing the growth pods on the digital scale. System height was collected using a Tailor's Tape. The malleability of the Tailor's Tape accommodated to the nonlinearity of stem propagation. Height was measured from the topsoil to the tip of the plant. The unevenness of the topsoil resulted in a ±0.2mm error factor for measured values. The leaf sizes were omitted from the data as there are no efficient methods of obtaining the leaf dimensions whilst also maintaining precision.

Measuring Soil pH Methodology

Soil pH was collected every-other-day using pH paper. Given that observations occurred under an FFD environment and pH paper based observations require determining the colour of the strip under non-coloured light, an issue arose. To combat this, the pH strips contacted the soil under FFD lighting and then pH determination occurred in a different room under normal/white lighting. With pH determination, estimates were rounded to the nearest '.0' or '.5'.

Collecting Biomass

Biomass is the weight of dried plant matter independent from soil and free/loose water. Collecting biomass was done only once and only at the end of the 40-day experimentation period. Firstly, the plants were removed from their growth pods and washed. They were then placed on a baking pan and put in the oven under low heat. Afterwards, when dried, some excess dirt was dusted off and then the gram biomass of the dried remains was collected.

RESULTS ANALYSIS AND DISCUSSION

Prefatory Notices

Foremost, the authors declare that No-Light data points are outliers due to their large difference in values compared to the illuminated sample data points.

All presented values are averages from both trials. To reiterate, the selected statistical methodology is mean-average. Millimeter measurements bear a factor of error of ±0.2mm. pH and temperature values are rounded to two decimal places because they are averages. Height measurements are from the topsoil to the tip of the plant.

Results

Five different factors contributed to the final data, of which are outlined as follows below.

1) Biomass/Dry Weight: overall on average, the monochromatic violet-light samples had the greatest final biomass of 43.24g, followed by monochromatic blue-light at 43.17g; No-Light samples had the lightest biomass of 16.56g. The biomass range is 43.24g (Violet) to 16.56g (No-Light). The average biomass for VL plants was 43.00g. (See Fig. 1) Point ‘P’ (peak) represents the greatest biomass value (violet-light) on Figure 1 and Point ‘T’ (trough) represents the smallest biomass value (No-Light).

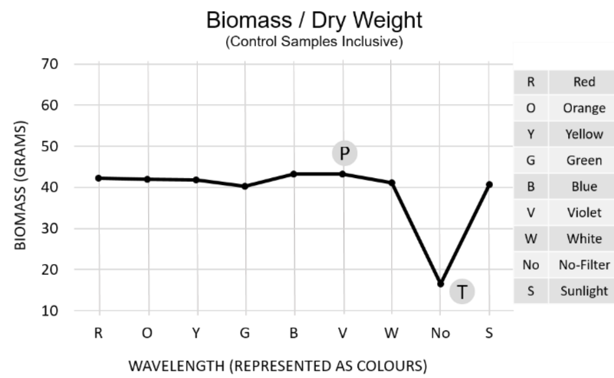

FIGURE 1

2) Final System Weight: overall on average, the monochromatic violet-light samples had the greatest overall system weight of 118.74g, followed by monochromatic blue-light at 118.67g; No-Light samples had the lightest overall system weight of 92.06g. The system weight range was 118.74g (Violet) to 92.06g (No-Light). (See Fig. 2) Point ‘P’ (peak) represents the greatest system weight value (violet-light) on Figure 2 and Point ‘T’ (trough) represents the lightest system weight (No-Light).

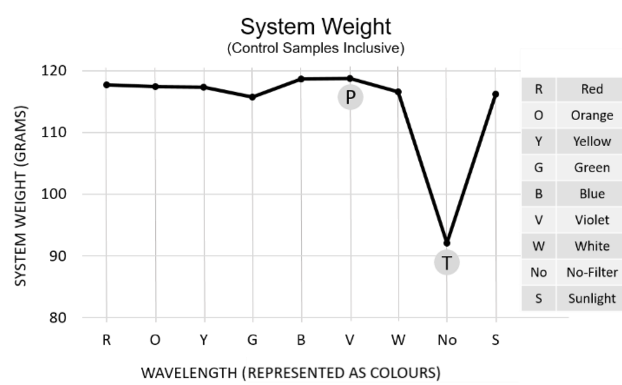

FIGURE 2

3) Plant Height: overall on average, the monochromatic violet-light samples had the tallest height of 288.7mm, followed by monochromatic blue-light of height 284.3mm; No-Light samples had the shortest height of 27.3mm. The height range is 288.7mm (Violet) to 27.3mm (No-Light). The average height for VL plants was 280.0mm. (See Fig. 3)

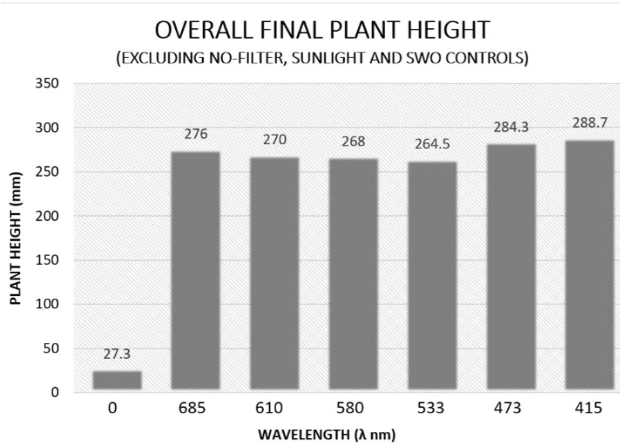

FIGURE 3

4) Soil pH: overall on average, the white-light samples had the most basic soil at 8.27, followed by monochromatic violet-light at 8.22; No-Light samples had the nearer-to-neutral soil at 7.70. The results pH range (see Fig. 4) is 8.27 (White) to 7.70 (No-Light), which is basic. The optimal pH range for *P. vulgaris* is 5.90 to 6.50 which is acidic. The former range is depicted on Fig. 4 as 'B' and the latter range is depicted as 'A'.

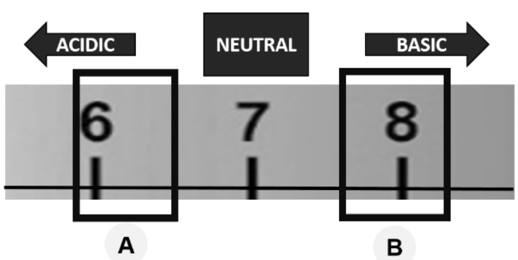

FIGURE 4

5) Soil Temperature: overall on average, the Sunlight samples had the warmest temperature of 20.66C, followed by white-light samples at 20.05C; No-Light samples had the coolest temperature of 17.52C. The temperature range (see Fig. 5) is 20.66C (Sunlight) to 17.52C (No-Light). The average temperature for VL systems was 19.38C. The boxed region in Figure 5 represents the soil temperature range.

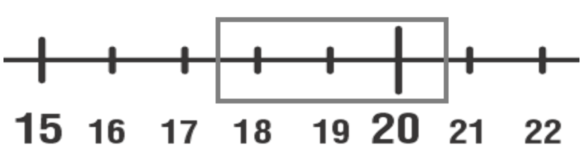

FIGURE 5

Analyzing Empirical Data

Biomass, System Weight, and Height are all interconnected because their values depend on physical plant growth. The results showed data points consistent with the hypothesis: plants exposed to wavelengths of higher energy were greater in biomass, weight, and height. In fact, violet-light samples had greater values than No-Solution and blue-light samples providing merit for the viability of the high quanta reasoning.

With regards to soil pH, samples grew in alkaline soil despite the fact that the optimal pH range of growth for *P. vulgaris* is acidic. The observed pH resilience, with integrations and research, can lead to developments regarding more resistant soil-biota.

Concerning temperature**,** the growth pods simulated greenhouses, maintaining internal temperatures within 20 Celsius which is the optimal temperature for *P. vulgaris*, especially when the external room temperature would drop to 16 Celsius during the winter time (of which was when the experimentations were conducted). The replication of this Greenhouse Effect within a closed system can be investigated as a means to combat and/or understand current global climate issues.

Controls Analysis: Final Appearance

The light sources affected the colour of plant parts, therefore No-Light and Sunlight controls were tested to keep with constancy and accuracy. These effects can be illusively understood with the following: No-Light samples had pale yellow leaves and white stems, consistent with light deficiency; Sunlight samples had darker-green leaves and red stems; LED-light samples had livelier-green leaves and green stems. Comparatively, the plants were all somewhat similar on colour-independent observations like height and weight. Conclusively, this is indicative of the fact that different sources of light, and the lack thereof of light, play a considerable role in the colour of the plant but not the yield.

Control Analysis: Yield and Germination

The presence of the solution in the system play an important role in the investigation of the hypothesis, therefore comparative No-Solution controls were tested to maintain constancy and accuracy. It was observed that samples with the solution present in their system (SWS) and systems absent of the solution (SWO) had different yields. The differences can be seen in the time till germination and the appearances of plant parts. SWO samples germinated after more than one week from planting (8 days on average) whereas SWS samples germinated within half a week from planting (3.5 days on average). SWO samples yielded plants with short and fragile stems with big leaves (50mm on average) while SWS samples yielded long and compact stems with medium-sized leaves (30mm on average).

Competitive Germination

Four *P. vulgaris* seeds, all uncracked and alike in weight and size, were planted per each individual system. It was observed during and at the end of the experimentation period that SWS samples sprouted four free stems with no branches whereas SWO samples sprouted only one free stem with an average three branches (with small flora that resembled weeds sprouting along the sides). It is theorized, therefore, that the presence of the solution within the system has an effect on the propagation of germination. More so to say, it is theorized that in SWO samples, only one of the four seeds will fully sprout and grow as if to compete for dominancy while the rest only pullulate as weed-like flora along the sidelines. Furthermore, the presence of this competitiveness is nonexistent in SWS samples.

Lessons Learned

Three important lessons were learned and they can be summated as follows: 1) information regarding low soil dependence, of which can be integrated to support pedological postulates, shows the ability of *P. vulgaris* to grow on average 28cm high and 43 grams heavy with soil just as much as that that could occupy a 100mL beaker. This can be applied for agronomic purposes in regions with low or poor earth; 2) the solution produced ammonia gas which elevated the soil pH but *P. vulgaris* grew nonetheless, far from its optimal (acidic) pH-range of 5.9 to 6.5. This information can be applied for agricultural purposes in regions where earth is acidic or basic; and 3) the results corroborated, with reiterated irrefutable and physical evidence, the strong relationship between the amount of CO2 and the rate of plant growth.

Conclusion

The results show that SWS short VL wavelength samples resulted in the greatest final biomass, system weight and height. The samples also proved resilient towards the non-optimal pH soil environment they were in for the entire duration of the experimentation period. Furthermore, a constant internal temperature was maintained within the system of said samples. This is definitive proof of accelerated photosynthesis and more developed adaptation to the environment, consistent with the claims of the hypothesis.

Given the evidential support for the hypothesis, it can be appropriately said that the final data supported the high quanta energy reasoning, due to the fact that monochromatic violet-light [violet-light samples] bested all the other wavelengths [other samples] that were tested.

It is also appropriate to say that 'SWS monochromatic LED-light'-based methodologies are a novel advantageous experimental technique for growing plants, especially during sunless occasions. Given the data, it is concluded that plant growth accelerated and further developed in LED-light exposed plants compared to Sunlight exposed plants, and there were more advantages in growing plants with the solution than without.

Developmental Applications

The outcomes present two important applications that, with further methodological investigation, can be efficaciously applied to help aid global issues. They are:

1) The utilization of elevated-CO2 techniques as the new, green, climate-change-fighting fertilizer. Conclusions made in the project regarding high CO2 concentrations within a closed system can be feasibly integrated to find a solution to rising GHG/CO2 emissions problems while simultaneously helping flora and fauna through supplying reactants for autotrophic

metabolism;

2) The application of new knowledge regarding successful plant yield with minimal and resilient soil into unconventional agronomy methods. The observed symbiotic relationship between plant and soil could, with further research and testing, lead to developments into a more tolerant, neutral, soil-biota supporting, fertile, porous, and/or aerated soil. This then could feasibly combat major agronomical issues faced by industries, thus benefiting both consumers and agro producers.

Improvements and Further Research

Something that merits consideration for improvement is using a different chemical buffer other than ammonium to prevent the production of ammonia gas.

Regarding further research, the experiment uses primary (R, B, Y) and secondary colours (O, G, V). Since secondary colours are combinations of primary colours, there could be a possible difference in results if instead two primary colours were used to simulate one secondary colour instead of just using a lone secondary colour filter (for example, instead of using a violet-light, a red- and blue- light could be used). To add, investigating the events of the reaction at the molecular level, using complex methods such as Quantum Yield, could further advance knowledge in quantum physics and related fields.

Acknowledgements

The authors extend their most sincere gratitude to Nancy Dionisio, Stephanie Finlay, Brooke Vandekerckhove, and Peter Slivinski of Sisler High School; Dr. Carl Bartels of the University of Manitoba; Dr. Kris Quinlan of the University of Toronto; and Hannah Dao & Christian Genaille for their advisory guidance and scholarly assistance in the project. ["Nothing would have been accomplished if not for their help." -ST]

Notices

"System" (as seen in terms like 'System Weight') represents the growth pod, solution, and plants all together. The solutions were replaced every week. Celsius is used synonymous to Centigrade. 'No-Filter samples' are used synonymous to 'White-light samples'. This project observes Systeme International units, both base and derived.

Abbreviations

&	and
CO2	carbon dioxide
C	Celsius/centigrade
cm	centimetre(s)
eV	electronvolt
FFD	filtered-flashlight-device
g	gram(s)
GHG	greenhouse gas(es)
J	joule(s)
LED	light-emitting diode
mL	millilitre(s)
mm	millimetre(s)
nm	nanometre(s)
P. vulgaris	*Phaseolus vulgaris*
pH	power of hydrogen
SWS	system with solution
SWO	system without solution
VL	visible light

Competing Interests

The authors have no conflicts of interest to declare.

BIBLIOGRAPHY

Works Cited

[1] Wang, Y., et. al. (2019). *Improve plant photosynthesis by a new slow-release carbon dioxide gas fertilizer* [Article]. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6648988/

[2] Gonzali, S., Kiferle, C., Perata, P. (2016). *Iodine biofortification of crops: agronomic biofortification, metabolic engineering and iodine bioavailability* [Article Abstract]. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/27835794

References

- Bushuyev, O., et. al. (2018). *What should we make with CO2 and how can we make it?* [Journal Article]. Retrieved from https://www.cell.com/joule/fulltext/S2542-4351(17)30076-4
- Clark, J. (2002). *The effect of catalysts on reaction rates* [Article]. Retrieved from http://www.chemguide.co.uk/physical/basicrates/catalyst.html
- Science Buddies Staff. (2017). *Presto! From black to clear with the magic of photochemistry* [Guidelines]. Retrieved from https://www.sciencebuddies.org/science-fair-projects/project-ideas/Chem_p095/chemistry/magic-of-photochemistry
- Humboldt State University Staff. (2015). *Electromagnetic spectrum* [Article]. Retrieved from http://gsp.humboldt.edu/olm_2016/Courses/GSP_216_Online/lesson1-2/spectrum.html
- Monnat Jr., G. (2012). *My first coral and clam* [Article]. Retrieved from http://www.austinreefclub.com/topic/22252-my-first-coral-and-clam/
- Hamilton, G., Sanabria, H. (2019). *Multiparameter fluorescent spectroscopy of single molecules: 6.2.3 Quantum yield* [Article]. Retrieved from https://www.sciencedirect.com/topics/chemistry/quantum-yield
- National Center for Biotechnology Information: PubChem. (2018). *Ammonium ion* [Database Article]. Retrieved from pubchem.ncbi.nlm.nih.gov/compound/Ammonium-ion
- National Center for Biotechnology Information: PubChem. (2018). *Oxalate* [Database Article]. Retrieved from pubchem.ncbi.nlm.nih.gov/compound/71081
- Didier, S. (2014). *What pH level do beans require to grow?* [Article]. Retrieved from https://homeguides.sfgate.com/ph-level-beans-require-grow-46747.html
- American Society of Agronomy. (2018). *Root exudates affect soil stability, water repellency* [Article Abstract]. Retrieved from https://www.sciencedaily.com/releases/2018/04/180418092033.htm
- Animated Clip Art of Plant (2007). Potted_Plant [Online Photograph]. Retrieved from https://www.aperfectworld.org/clipart/plants/potted_plant.png