Biocem experimnt

Science, Genetics



Experiment 1: Quantitative assessment of Some Cellular Constituents Summary: Euglena gracilis are unicellular organisms in the Protist Kingdom. They are known to have both plant and animal characteristics. Although, Euglena cells contain a variety of cellular constituents, their cellular constituents should be presented in equal ratio. The objective of this experiment is to determine Euglena's cellular components in cells and then to establish their cellular constituents by comparing the experimental results to the expected concentrations given on pg 10 of the lab manual. Concentration of cells in stock culture used for extraction = $9.0\tilde{A}$ -105 cells/ml Total number of cells used for extraction = 4. 05 \tilde{A} — 107 cells Color of Band | Suspected Chlorophyll Pigment | Distance Moved from Origin (cm) | Calculations | Rf Value | Yellow | Violaxanthin/Neoxanthin | 0.7 | 0.7cm/5. 3cm | 0. 132 | Light Green | Chlorophyll b | 0. 9 | 0. 9cm/5. 3cm | 0. 170 | Green | Chlorophyll b | 1. 1 | 1. 1cm/5. 3cm | 0. 208 | Light Yellow | Lutein & Zoaxanthin | 1. 6 | 1. 6cm/5. 3cm | 0. 302 | Light Green | Chlorophyll a | 2 | 2. 0cm/5. 3cm | 0. 377 | Yellow | Carotene | 5. 1 | 5. 1cm/5. 3cm | 0. 962 | Results: 1) Estimation of Cell Number: The cell concentration of Euglena sample was determined in order to calculate their cellular constituents. 5 ml sample taken from the stock culture was used to calculate the cell number. The initial absorbance of the Euglena sample measured on the Spectrophotometer 2000 at 675 nm was 2. 410 (beyond the maximum allowed abs. of 0. 6). The 5 ml sample was diluted with 30 ml water. After a dilution factor of 7 was applied, the absorbance of the sample was brought down to 0. 547. By finding the absorbance, the cell concentration can be determined via the Euglena turbidity curve on page 14. An absorbance of 0.

547 correlated to a cell number of approximately 9. 0×105 cells/ml. The total number of cells used for extraction was: (9. 0×105 cells/ml) × (45ml used for extraction) = 4.05 \tilde{A} — 107 cells. 2) Thin Layer Chromatography: a) Chlorophyll Concentration Determination: Conc. (mg/ml) of total chlorophyll in an acetone extract = Abs. at 652nm / 36 \AA - amount of pooled acetone extract Conc. (mg/ml) of total chlorophyll in an acetone extract = (0. 253 / 36)15 = 0.105 mg/ml To find the total chlorophyll per cell, the total chlorophyll in acetone extract was multiplied by the dilution factors and then was divided by the total number of cells per ml in the stock sample. $(0.105 \times 100 \text{ s})$ 7 mg/ml) / (4. 05 \tilde{A} — 107 cells/ml) = 1. 81 x 10-8 mg chlorophyll/cell b) Carotenoid Concentration Determination: Because both carotenoids and chlorophyll are found in the acetone solution, certain correction factors must be applied to the spectrophotometry in order to negate the presence of chlorophyll. Even though carotenoids and chlorophyll have their maximum absorption at 480nm, chlorophyll absorbs in the red region of the spectrum while carotenoids do not. By taking that and the molar extinction coefficients into account, the correction factors effectively eliminate the contributions of chlorophyll. Corrected Abs. at 480 = Abs. at 480nm — [0. 114 x (Abs. at 663nm)] — [0. 638 x (Abs. at 645nm)] Corrected Abs. at 480 = 0. 498 — [0. 114×0.422 - [0.638 x 0.164] Corrected Abs. at 480 = 0.345 Total conc. (mg/ml) of carotenoid in an acetone extract= Corrected Abs. at 480 / 250 Åamount of pooled acetone extract Total conc. (mg/ml) of carotenoid in an acetone extract = $(0.345 / 250)15 = 2.07 \times 10-2 \text{ mg/ml}$ (2.07 x 10-2 x 7) mg/ml) / (4. 05 \tilde{A} — 107 cells/ml) = 3. 58 x 10-9 mg carotenoid/cell 4) Analysis of Supernatant F — DNA: Amino acids, sugars, nucleotides, acid

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soluble phosphate compounds, ethanol soluble lipids, fatty acids, glycerophosphatides, sphingolipids, steroids, and ether soluble lipids were extracted sequentially from pellets A-D of Euglena cells. Pellet E was left to dry with the help of hot water since it contained DNA, RNA, and proteins. Afterwards, 5% PCA was added to the tube containing pellet E, extracting DNA and RNA. Supernatant F contained hydrolyzed nucleic acids; DNA and RNA. In order to determine the concentration of DNA in supernatant F, the Dische dipehnylamine reaction was applied. In the Dische reaction, diphenylamine reacts with DNA in a heated acidic solution, forming a blue colored complex with deoxypentose of the hydrolyzed DNA nucleotide. Although supernatant F also contained RNA, it won't impact the results because diphenylamine is highly specific for mono and dideoxypentose. The blue colored complex will allow the DNA to be measured at different concentrations at 600nm. As shown on table 3, 7 tubes and with different solutions were prepared for this procedure. Based on the trendline equation of graph 2, the unknown DNA concentrations of the solutions can be determined. In order to find the unknown concentration of the DNA, the equation y = mx + b must be used. According to the graph, y = 1.861x + 0. 005 with m = 1. 861 and b = 0. 005. X can be solved by plugging y (absorbance at 600nm) into the rearranged equation of x = (y - 0.005) / (1.861). Table 5: DNA Concentrations of Supernatant F Solutions TestTube # | Absorbanceat 600nm | Vol. SupernatantF (ml) | DNA Concentration (mg/ml) | $4 \mid 0.183 \mid 0.25 \mid x = (0.183 - 0.005) / (1.861)x = 0.0956 \mid 5 \mid 0.286 \mid 0.$ 50 | x = (0.286 - 0.005) / (1.861)x = 0.151 | 6 | 0.510 | 1.00 | x = (0.510)-0.005 / (1.861)x = 0.271 | DNA Concentration Determination: The

amount of DNA present per cell can be calculated by finding the average DNA concentrations of the 3 solutions. But since the absorbance of test tube 6 didn't fall within range, only the DNA concentrations of test tube 4 and 5 will be averaged. [(0. 0956mg/ml / 0. 10ml) + (0. 151mg/ml / 0. 25ml)] /2= (0.780 mg/ml) 4 = 3.12 mg/ml After considering the dilution factors, the total number of cells in the stock culture was: (4. 05 \tilde{A} — 107 cells/ml) Amount of DNA per Cell = (Total DNA of Unknown Solution) / (Total # cells/ml stock) Amount of DNA per Cell = (3. 12mg/ml) / (4. 05 \tilde{A} — 107 cells/ml) = 7. 70 \tilde{A} — 10-8 mg DNA per cell (5) Analysis of Supernatant F — RNA: Orcinol reaction is used to determine different RNA concentrations presence in supernatant F. The Orcinol reagent, contains 40 ml 12N HCl, 1 ml 10% FeCl3. 6H20 and 10 ml of 1% orcinol solution, interacts with RNA to form a green colored complex. The absorbances of Orcinol reagent with different RNA concentrations were taken at 670nm. Table 6 shows the solutions prepared for RNA concentration analysis. Table 9: Contents of Solutions Prepared to Determine Protein Concentration Test Tube # | Vol. of Proteinstandard (ml) | Vol. of Sampleof Pellet F (ml) | Vol. H20 (ml) | Vol. Biuret (ml) | 1 | 0. 25 | 0. 0 1.75 5.0 2 0.50 0.0 1.50 5.0 3 0.75 0.0 1.25 5.0 4 2. 00 | 0. 0 | 0. 00 | 5. 0 | 5 | 0. 00 | 0. 0 | 2. 00 | 5. 0 | 6 | 0. 00 | 0. 5 | 1. 50 | 5. 0 | 7 | 0. 00 | 1. 0 | 1. 00 | 5. 0 | 8 | 0. 00 | 1. 5 | 0. 50 | 5. 0 | 9 | 0. 00 | 2. 0 | 0. 00 | 5. 0 | Note: Protein Standard Concentration: 5 mg/ml In order to create different RNA standard concentrations, dilution was made by adding water to protein standard; while keeping the same total volume. The absorbance values of each test tube were taken at 545nm. The results of the experiment were very convincing. From this experiment, I found that the

ratios of chlorophyll: carotenoids: DNA: RNA: protein in Euglena cells were 1. 000: 0. 198: 4. 254: 1. 950: 37. 017, respectively. The standard ratios of chlorophyll: carotenoids: DNA: RNA: protein are 1. 00: 0. 60: 5. 00: 38. 00: 446.00, respectively. By comparing the results, one will agree that the experimental order of abundance of each component is similar to that of the standards', with the exception of RNA. Other than that the outcome of the experiment was pretty much consistence with my prediction. RNA is the byproduct of DNA transcription and is central to the synthesis of protein. In principle and as shown by the standard ratio, RNA is suppose to be roughly 8 times more abundance than DNA. However, what I obtained, the ratio of RNA: DNA is only 1. 950: 4. 254, respectively. A few possible explanations might be that RNA sample was not prepared correctly during the Orrcinol reaction procedure or that the absorbance of the RNA concentrations wasn't taken properly, leading to a lower value. Another possibility might be that the RNA sample was left at room temperature for too long before the start of the procedure and so some RNA denatured. The result of the TLC Plate was better than I expected it. Not only were six color bands observed, but also the suspected chlorophyll pigments were easy to identify based on the color and the distances moved from origin. This shows that chlorophyll pigments were extracted with the right amount of acetone or that the Euglena cells contained a good amount of chlorophyll pigments. The DNA standard curve appeared to be good, with a decent looking trendline and a regression value very close to 1. However, the standard curves for RNA and protein appears conflicting, with the trendline starting at absorbance above zero. The logical

reason for this inconsistency might be that different reagents used in RNA and protein analysis altered the reading.