

# [Determining osmotic potential using density gradient](https://assignbuster.com/determining-osmotic-potential-using-density-gradient/)

A cell, when exposed to an environment where the external water potential is less negative than the internal water potential, will loose water by osmosis down a concentration gradient. Conversely, when exposed to an environment where the internal water potential is less negative than the external water potential the cell will take in water from the surrounding solution. In the case of the former this has the effect of the cell loosing its ability to exert pressure on the cell wall and become flaccid. If water loss is consequential the cell will eventually plasmolyse. The point at which the cell is neither turgid nor flaccid and the net movement of water has reached dynamic equilibrium is known as insipient plasmolysis. It is at this point that the osmotic potential of the cell is equal to the osmotic potential of the surrounding solute. In a more concentrated solution, plasmolysis will continue, causing the protoplasm to pull away from the cell wall leaving a space which gradually fills with the external surrounding fluid. As the osmoticum enters the gap between the protoplasm and the cell wall, the cell density increases. Because the osmoticum of sucrose is denser than water, the plasmolysed cell is therefore denser than the non plasmolysed cell and will travel further and at a quicker rate through a density gradient.

Aims

To construct and utilise a density gradient to plot a graph from which the point of insipient plasmolysis can be ascertained, and hence the osmotic potential of a plant cell found.

Method

As script – A mean of two values was taken as time did not permit for the experiment to be run three times.

A graph was then plotted of the mean distance travelled by each stem section against the molar concentration in which it had been equilibrated. The graph was then analysed to see at which point the gradient changed significantly and the point of insipient plasmolysis was found by interpolation thus giving the osmotic potential of the cell.

Results

Figure 1 shows the stems fell at a steady rate in a gradual decline until the 0. 3m point where the graph dips sharply to the 0. 2m point. Suggesting that the point of insipient plasmolysis is around 0. 2m as the steep change in direction to the 03. m point implies that the cells have increased in density thus travelling further and more quickly. The readings at 0. 1m do not fit the general trend of the graph suggesting that they are anomalies in the data.

Discussion and Evaluation

The change in the graph occurs because cell membranes in the tissue start to pull away from the cell walls, at the 0. 2m concentration. At the 0. 3m solution point, more water has left the cells by osmosis in an attempt to achieve equilibrium in the surrounding fluid, however in doing so the cells have become plasmolysed, allowing the sucrose solution to enter the space between the cell membrane and cell wall, therefore it is here the initial increase in density is seen as a sharp increase in the distance travelled by the stem sections. As the cells become further plasmolysed due to immersion in increasing extracellular concentrations, more sucrose solution enters the space in the cells causing them to become denser and hence the stem sections travel further. Insipient plasmolysis was shown to occur when the stems were equilibrated in 0. 2 molar sucrose solution; hence because the solute potential of a solution is proportional to its molarity (Campbell Reece et al.) the osmotic potential of the solution was 0. 2 moles. At the point of insipient plasmolysis the osmotic potential of the cell is equal to the osmotic potential of the surrounding fluid and therefore the osmotic potential of a plant cell is 0. 2moles. The readings taken for the stem in the 0. 1 molar solution show that the stem travelled quite some way, this should not have occurred as the cells should not have started to plasmolyse and they should in fact have been turgid at this point as the osmotic potential of the cell is 0. 2m and as such has a less negative water potential than the surrounding fluid, encouraging uptake of water into the cell from the surrounding fluid. The stems were prepared in the group it may have been that the stems were not uniformly cut and possibly weighed heavier in the first instance. It would have been more prudent to run the experiment a few more times to gain a more accurate mean for the readings. However, the readings obtained are sufficient to produce a graph from which we can identify the point of insipient plasmolysis.

Conclusion

The Osmotic potential of plant cells is equal to that of insipient plasmolysis which is, 0. 2moles

## References:

Campbell, R., Reece, J., Urry, L., Cain, M., Wasserman, A., Minorsky, P., and Jackson, R. (2008) Biology, 8th edition, Pearson International: Benjamin Cummings

Bibliography

Bioskills Practical book

Enzyme Hydrolysis of Glycogen by Alpha and Beta Amylase

Introduction

After a meal carbohydrates are stored in the liver as Glycogen. Glycogen is a branched polymer of glucose where glucose residues are linked by alpha 1-4 glycosidic bonds in linear chains and branched points are linked by alpha 1-6 glycosidic bonds. When required this Glycogen is released back into the bloodstream but first needs to broken down into smaller ‘ usable’ disaccharides. Alpha amylases catalyse the hydrolysis of glycogen at the 1-4 linkages, producing Maltose and Maltotriose. Beta amylase also acts in the same manner, but only acts at the non reducing end of the polysaccharide as it is an exo-amylase. Once a branch is reached a limit dextrin is produced as hydrolysis stops. Glycogen digestion by enzymes can be ascertained by determining the amount of product produced during hydrolysis. The resulting product being a reducing sugar, which reduces yellow DNS dye to produce an orange red colour (3-amino-5 nitrosalicylic acid). The more reducing sugar produced, the darker and denser the colour produced during the reduction reaction. A spectrophotometer is used in order to measure the density of the resulting solution as density increases so does absorbance at 540nm.

Aim

To determine which if any of two enzymes, Alpha and Beta Amylase digests glygogen most efficiently.

Method – As Script

Maltose concentrations were converted into micromoles per ml and a calibration curve was constructed. A regression line was added and an equation for the line found which was used later in order to find concentrations for each enzyme after the assay had been run and absorbance’s found. These concentrations were then plotted on a separate graph and the graph analysed to ascertain which enzyme performed most efficiently.

Results

The results in Figure 3 show that alpha amylase yields the most product reaching over 2. 5 micromoles over time but the graphs also show a similar curve suggesting that the reaction for both enzymes is progressing at a similar rate.

Discussion

If a gradient is taken for the initial activity for both enzymes it is found that they both produce 0. 1 micromoles of product per ml per minute and hence the rate of reaction appears to be the same for both enzymes. However alpha amylase clearly produces more reducing sugar, due to its reaction within the glycogen compound and the initial rate must therefore be faster than that of Beta amylase which only reacts at the reducing ends of the polysaccharide and is also inhibited by its own product maltose. (www. homedistiller. org/enzymes 11. 4. 10) This suggests that t0 is not t0, as suggested.

During the experiment the alpha amylase gave absorbance readings at 540nm at over 1 as did the maltose during the making of the calibration curve, as the absorbance of radiation at a particular wavelength by a solution is ‘ directly proportional to the concentration of the absorbing solute’ the readings over 1 are highly likely to be inaccurate as the linear relationship only applies up to a certain concentration, and above this concentration the relationship becomes non linear. As can be seen in figure 2 most of the absorbances for alpha amylase were over one and as such should be questioned as to their validity.

On this basis the alpha amylase should have been diluted further to give absorbances of less than 1 and then this multiplied by the dilution factor to give the absorbance of the original solution.

From the curves in Figure 3 it is very apparent that t0 is not t0 and the majority of the reactions in both cases took place almost instantaneously. To find t0 further experimentation should be carried out during the time the curve represents a zero order reaction. I. e. where the rate is constant with time. The substrate concentrations should be the variable factor with multiple readings taken, and the velocity measured for each one. This data should then be plotted and the two parameters which define enzyme kinetics, Km and V max found. This information can then be applied to the Lineweaver-Burke model and the point at which the line crosses the y axis is the point of 1/V0. This figure can then be differentiated to find t0.

Conclusion

It would appear the alpha amylase is the most efficient enzyme for digestion of glycogen.