# Effect of ph and glucose on plant growth

**Abstract:**

An experiment was designed and conducted to investigate the population growth of the yeast Saccharomyces cerevisiae under various environment conditions such as temperature, pH levels and glucose concentration. The research questions were then arrived as: What is the effect of differing temperatures on Saccharomyces cerevisiae population growth?

What is the effect of differing pH levels on Saccharomyces cerevisiae population growth?

What is the effect of differing glucose concentrations on Saccharomyces cerevisiae population growth?

The different temperatures were chosen based on kinetics and each temperature differing from the other by at least 10oC, so a notable change in the yeast population to be observed. Two of the temperatures chosen were below the optimum temperature and two above and one in the optimum temperature.

Based on the optimum pH levels for the growth of the yeast, certain buffers with two pH values above and two below of the optimum pH and one in the optimum pH were prepared and stored.

The glucose concentration that was used in cultures which tested for the effect of temperature and pH was chosen in such a way that would enable the yeast population to grow without limitation as far as glucose is concerned. One of the options for testing the effect of glucose over the yeast growth was the absence of glucose from the culture. The other options were

to halve the optimum glucose concentration and the last was higher of the optimum value.

When testing the different temperatures, the results showed that there was little growth in relative low and high temperatures and very high growth in the optimum temperature (the population almost quadrupled). In the different pH levels the yeast growth was little in low and high pH levels but was increased as pH was reaching the optimum pH. In the case of different glucose concentrations, the results showed that with no glucose in the culture was a small growth; in the glucose concentration of halve of the optimum value there was growth but again less than the optimum; in the glucose concentration above optimum there was very high growth as there was in the optimum value.

# Chapter 1: Introduction

**Research Questions:**

- What is the effect of differing temperatures on Saccharomyces cerevisiae population growth?
- What is the effect of differing pH levels on Saccharomyces cerevisiae population growth?
- What is the effect of differing glucose concentrations on Saccharomyces cerevisiae population growth?

**The yeast:**

Saccharomyces cerevisiae is a single celled fungus that reproduces asexually by budding or division. It is one of the most well studied eukaryotic model organisms in both molecular and cell biology.

Saccharomyces cerevisiae is maybe the most important and used fungus in the history of the world even from ancient times because of its use in the brewing of beer and in rising of dough in bread. That is the reason why is called brewer’s yeast and baker’s yeast, due to the use of different strains of Saccharomyces for the alcoholic and sugar fermentation.

S. cerevisiae is a very good type of yeast for biological studies owing to the rapid growth (doubling time 1. 5-2 hours at 30 °C), the dispersed cells and the ease of replica planting. Moreover is a non-pathogenic organism, so can be handled fearlessly with only little precautions. Also large amounts of commercial baker’s yeast are available with result being an easy and cheap source for biochemical studies.

S. cerevisiae has round to ovoid cells between 3-8Î¼m in diameter

**Respiration:**

In biology, respiration is defined as: “ the process by which the energy in food molecules is made available for an organism to do biological work” (Kent, 2000; p. 100). It is also called Cellular respiration. This process of cellular respiration happens in every living cell as it is the only way to obtain energy in a form that will be usable for the cell, so it can carry out the functions of movement, growth and reproduction (ibid).

The food in yeasts must be obtained as they cannot produce it on their own. For yeasts, a very good source of energy is sugars. All strains of S. cerevisiae can metabolize glucose (a hexose sugar), maltose and trehalose.

**Adenosine Triphosphate (ATP):**

Adenosine Triphospate known also as ATP is the form of chemical energy that cells use to carry out biological activities. Without ATP an organism can't survive. During cell respiration the energy that is found in food molecules is transformed to ATP (Kent, 2000; p. 100).

## Types of Respiration:

There are two main types of respiration that take place within a cell: Anaerobic respiration (without oxygen) and Aerobic respiration (with oxygen). S. cerevisiae can metabolize sugars in both ways, but in this research the cultures of yeast were exposed to air hence to oxygen, so aerobic respiration was mainly the way that yeast cells grew and reproduced.

## Aerobic Respiration:

Aerobic respiration is a complex process which involves different steps of reactions and its purpose is to metabolize food molecules. As these reactions take place and food is broken down, energy is released which is then used to synthesize ATP from ADP (Adenosine diphosphate) and inorganic phosphate (Kent, 2000; p. 101). These reactions are carried out by special enzymes. There are the three major metabolic stages in aerobic respiration: glycolysis (which is also part of anaerobic respiration), Krebs cycle, electron transport chain and oxidative phosphorylation.

Krebs cycle: The central phase of the aerobic respiration and occurs in the mitochondrial matrix. It involves the production of acetylcoenzyme A (acetyl-CoA) (Kent, 2000; p. 104).

Electron Transport Chain: It involves the highest production of ATP during respiration, meaning the 90% of ATP is produced in this stage. This metabolic stage occurs in the inner mitochondrial membrane (Greenwood. et al. 2007; p. 127).

**Glycolysis:**

Cell respiration has to do with the production of ATP by the oxidation of sugars, fats or other substrates. In this research as substrate was used glucose. When glucose is the substrate, the first metabolic pathway of cell respiration is glycolysis, which is carried out by enzymes in the cytoplasm of the cell. A small amount of ATP is produced in this pathway by the oxidation of glucose. Glycolysis consists part of aerobic and anaerobic respiration because no oxygen is used (Allot, 2007; p. 73).

**Enzymes:**

Thousands of chemical reactions are carried out within a cell. These reactions most of the times occur in a very slow rate. For that reason living organisms make biological catalysts which are called enzymes and speed up these reactions. “ Enzymes are globular proteins which act as catalysts of chemical reactions” (Allot, 2007; p. 18). An enzyme can increase to more than a billion of times the rate of a chemical reaction. Also cells can control which reaction occurs in their cytoplasm by making some enzymes and not others. Enzymes achieve to increase the rate of a reaction by decreasing the activation energy (the minimum amount of energy required for a reaction to occur) (Green. Et al. 2008; p. 167)of the substrate or the substrates, when binding to the activation site (“ is the part of the enzyme’s surface into which

the substrate is bound and undergoes reaction") (Greenwood. et al. 2007; p. 114)

Enzymes are sensitive molecules with very specific structure which enables them to carry out specific reactions. This structure including the active site can be damaged by various conditions and substrates. This damage is called denaturation and is usually permanent for an enzyme and if denaturation is occurred the enzyme can no longer carry out its function. As a result when enzymes are required to catalyze a reaction, is necessary that they have appropriate conditions. It should be remembered that different enzymes have different ideal conditions. The factors that affect the enzyme activity are: the temperature, the pH, the substrate concentration. In a specific point for each of the previous factors, enzymes work in the most effective way, known as optimum conditions.

The effect of temperature, pH and substrate concentration upon the enzyme activity which affects the growth of S. cerevisiae yeast cells are studied in this research.

**Effect of Temperature:**

As the temperature is increased in an enzyme-catalysed reaction, the rate of reaction is increased up to maximum in a specific temperature. This is called optimum temperature. The optimum temperature of Saccharomyces cerevisiae is 30o- 32oC.

In temperatures below of the optimum, when increasing the temperature there is an increase in the kinetic energy of the reactants and there are more

frequent collisions between the active site and the substrates, so the activity of the enzymes is increased.

The rate still rises as the temperature increases; till it reaches the highest rate where is the optimum temperature hence the highest enzyme activity.

Above this temperature the rate starts to drop rapidly. This is due to the high energy that causes vibration inside the enzyme with result the bonds which maintain the structure of enzyme to break. This causes denaturation and the active site can no longer fit the substrate.

Overall, at very low temperatures the enzyme activity hence the rate is low due to the low kinetic energy of the substrate but there is no denaturation, at the optimum temperature the rate is the highest and levels off because the increase in kinetic energy of substrate is cancelled out by the denaturation of the enzyme and at high temperatures enzymes are denaturated and the rate falls dramatically because denaturation exceeds the high kinetic energy of the substrates. These are summarized in the following graph.

**Effect of pH (hydrogen ion concentration):**

Most of the enzymes operate effectively in a small range of pH values. Between these pH values there is an optimum pH value in which the enzyme activity is the highest. The optimum pH of Saccharomyces cerevisiae is 5. 5. Acids and alkalis cause denaturation of the structure of the enzyme by breaking mainly hydrogen and ionic bonds with result the substrate can’t fit the active site. Furthermore the charges of the amino acids within the active site are affected by pH changes, so the enzyme is not able to form an

enzyme-substrate complex. Above and below the optimum pH the enzymatic activity hence the rate is reduced considerably.

### Effect of Substrate concentration:

In an enzyme-catalysed reaction the rate increases in direct proportion to the substrate concentration. The optimum glucose concentration of Saccharomyces cerevisiae is 2%. At low substrate concentrations, the rate of enzymatic activity increases sharply as the substrate increases. This occurs due to the more frequent collisions between the substrate molecules and the unoccupied active sites. On the other hand, at high substrate concentrations the biggest part of the active sites have been occupied with result when increasing the substrate concentration there is little effect on the rate of enzymatic activity.

# Chapter 2: Methodology

## Objectives of the study:

To determine how the different temperatures affect the growth of population of S. cerevisiae.

To determine how the different pH values affect the population growth of S. cerevisiae.

To determine how the different glucose concentrations affect the population growth of S. cerevisiae.

## Hypothesis:

Hypothesis 1: The population of S. cerevisiae will grow the most at the optimum temperature, meaning between 28oC to 32oC, and also the

population growth at temperatures below the optimum will be higher than the population growth at temperatures above the optimum.

Hypothesis 2: In the optimum pH, meaning at low acidic conditions of pH 5. 5 to pH 6, there will be the highest S. Cerevisiae yeast cell population growth. At pH levels above and below the optimum pH there will be less growth but this growth level will be relatively of the same degree for the values of pH above and below.

Hypothesis 3: In the optimum glucose concentration, meaning about 2% glucose, will occur the highest yeast growth. In the glucose concentration below of the optimum there will be much lower growth, whereas in the absence of glucose there will be almost none yeast growth.

**Variables:**

When testing the effect of differing temperatures on S. cerevisiae population growth:

Independent variable: Temperature (5o C, 15oC, 30oC, 50oC, 60oC).

Dependent variable: Number of S. cerevisiae cells.

Controlled variables: 7mL buffer of pH 6 in every test tube, glucose concentration 2mL (2% glucose solution) in every test tube and 1mL yeast (0. 02% yeast solution) in every test tube.

When testing the effect of differing pH levels:

Independent variable: pH (3, 4, 6, 8).

Dependent variable: Number of S. cerevisiae cells.

Controlled variables: Temperature (30oC), glucose concentration 2mL (2% glucose solution) in every test tube, 7mL buffer in every test tube, 1mL yeast (0. 02% yeast solution).

When testing the effect of differing glucose concentrations on S. cerevisiae population growth:

Independent variable: Glucose concentration (0mL, 1mL, 2mL, 3mL of 2% glucose solution each).

Dependent variable: The number of S. cerevisiae cells.

Controlled variables: Temperature (30oC), 9mL buffer of pH 6 at 0mL glucose, 8mL buffer of pH 6 at 1mL glucose, 7mL buffer of pH 5. 5 at 2mL glucose, 6mL buffer of pH 6 at 3mL glucose, 1mL yeast (0. 02% yeast solution) in every test tube.

**Materials/ Apparatus:**

- Test tubes
- Burette
- Micropipettes
- Pipettes
- Pipette-fillers
- Graduated cylinder of 10mL, 250mL and 1000mL
- Volumetric Flasks of 250mL and 1000mL
- Funnels
- Spatula

- Weight boats
- Beakers
- Plastic wash bottles
- Plastic bottles
- Cover slip
- Haemocytometer
- Microscope
- Digital multi-log
- Balance
- Waterbath
- Magnetic stirrer
- Thermometer
- Ethanol 70%
- 0. 1M Citric acid
- 0. 2M Sodium hydrogen phosphate
- Distilled water
- Yeast: Saccharomyces cerevisiae

Source of yeast: YIOTIS S. A, INDUSTRY OF NUTRITIONAL PRODUCTS, ATHENS, GREECE.

## Procedure:

### Day 1:

The first step before the start of the aerobic fermentation of yeast was to prepare the buffers. For the preparation of buffers of different pH, citric acid (3-carboxy-3-hydroxypentanedioic acid) and sodium hydrogen phosphate ($Na_2HPO_4$) were used. Four plastic bottles, labeled each with one pH value

(3, 4, 6, 8 respectively), were required. 100mL of each of the buffers were prepared.

The stock solutions of citric acid and Na2HPO4 firstly prepared.

For the preparation of stock solution of citric acid of concentration 0. 1M and volume 1L, 19. 2g of citric acid and 1L distilled water required.

For the preparation of stock solution of Na2HPO4 of concentration 0. 2M and volume 1L, 28. 4g Na2HPO4 and 1L distilled water required.

A balance and a weigh boat required for the measuring of masses. The solutions were added and stored in two volumetric flasks of 1L respectively, which measured the volume of distilled water. Citric acid and Na2HPO4 were added into the flasks with the aid of funnels.

The volumes were measured and put into four different plastic bottles by using two burettes of 50mL. The validity of each pH value checked by using a digital multi-log.

The next step was to prepare the glucose solution. For the preparation of glucose one volumetric flask of 500mL used to measure the volume of distilled water and to store the glucose solution. 10g of glucose were weighed by using a balance, a weigh boat and a spatula. Half of a 100mL beaker filled with distilled water was used to dissolve the 10g of glucose. A magnetic stirrer used for better dissolution. After glucose was complete dissolved, was added to the 500mL flask using a funnel. The rest of the flask was filled up to 500mL with distilled water.

Then, the yeast solution prepared for the purpose of the experiments of that day. Every day a new yeast solution was prepared. For the yeast solution 0. 10g of dry yeast were weighted from sachet with a spatula and placed on the weight boat. The yeast was added to a 1000mL volumetric flask filled with 500mL distilled water with the aid of a funnel in order to avoid staking of dry yeast in the cylindrical walls of the flask. Afterwards the solution was swirled by smooth shaking.

After everything was ready the experiments for the studying of the effect of differing temperatures on S. cerevisiae growth initiated. Three water baths were prepared and each one adjusted in three different temperatures 30oC, 50oC and 60oC. Each temperature was tested by using a thermometer and a digital multi-log sensor. Two refrigerators were used for the low temperatures and adjusted at 5oC and 15oC. After all temperatures have been reached, the preparation of cultures started. Five test tubes labelled with one temperature each. The cultures were prepared with half an hour difference in order to test the stability of the temperature and to take a sample from each test tube and count the initial population. A pipette of 25mL used to introduce the glucose to the test tube. A 10mL graduated cylinder used to measure the volume of the buffer and then was introduced into the test tube also. Then with another 25mL pipette, 1mL yeast was taken and placed also into the test tube. The yeast solution was shaken before taking the sample as yeast cells tend to sink to the bottom of the flask due to their weight. Afterwards by using a micropipette, a sample was taken from the culture inside the test tube and placed on haemocytometer

and then to the microscope to count the initial population (the cells found in the borders of the chambers were counted).

The haemocytometer is a specialised microscopical apparatus used to count cells and other organelles. A haemocytometer consists of two counting chambers. Each chamber consists of an arrangement of squares of different sizes which are used to count easily the cells. These squares of different size form different grid layouts. In the centre of each chamber it is found a grid of squares of 0. 2mm 0. 2mm 0. 1mm dimensions. There is another grid of squares of dimensions 0. 25mm 0. 25mm 0. 1mm, in each of the four corners around the central grid. The grids of squares of 0. 25mm 0. 25mm 0. 1mm dimensions were used for the counting of the yeast cells. A cover slip is placed above the chambers, so the samples are spread equally due to capillary action on the counting area.

The test tube was then placed for 24hours in the temperature corresponding to what was labeled. This procedure was the same for the rest four test tubes. In the end of the day the glucose solution 2% was placed in the refrigerator, the 1000mL flask with the yeast solution, the haemocytometer, the cover glass and all the other apparatus was cleaned with ethanol 70% and washed with distilled water and left to dry. The use of 70% ethanol for the cleaning of haemocytometer doesn’t have any negative effect on the yeast cells that were place on it to be counted. This happened in the end of every day.

**Day 2:**

The next day each test tube was removed with half an hour difference in the order that they were left for fermentation. Then a sample was taken with the use of a micropipette and placed on haemocytometer and again to microscope to count the yeast cells.

After finishing with temperature testing the next thing was to study the effect of pH levels on S. cerevisiae population growth.

A yeast solution was prepared the same way as Day 1. The glucose solution was removed from the refrigerator. Clean test tubes taken and labeled with different pH values 3, 4, 6, 8. A water bath adjusted at 30oC. Again, every culture was prepared the same way as Day 1 and placed in a test tube with half an hour difference. All test tubes with different pH levels were placed in the same water bath for 24hours. Before each test tube was placed in water bath, a sample was taken to count the initial population of each.

**Day 3:**

The cultures were removed in the order that were left to ferment and samples were taken to count the yeast population from each one. Between each measurement the haemocytometer was cleaned as was mentioned in Day 1.

Finally, the effect of glucose concentration on yeast population growth was left. New yeast solution was prepared. The water was adjusted at 30oC. In clean test tubes the new cultures were prepared to test the glucose concentrations. The test tubes were labelled each with one concentration

value. Samples were taken from each to count the initial population. The cultures were placed in water bath to ferment.

**Day 4:**

The cultures were removed from water bath and samples taken to count the yeast population.

Weaknesses and Improvements:

Weakness

Improvement

In the populations of yeasts cells that were counted in the microscope, there were both alive and dead cells or denaturated cells.

A dye such as methylene blue could be used to determine in each counting the live and the dead or inactive cells. The cells which would remain colorless would indicate enzyme activity and the dead or denaturated cells would be turned into blue.

Methylene blue should be used only after the fermentation has finished because it inhibits the yeast cells by consuming the hydrogen ions that are produced during respiration.

The test tubes, where the yeast cultures were left for fermentation, were slightly closed on the top with cotton in order to prevent the entrance of other microorganisms. This cotton plug prevented the easy flow of fresh air (containing oxygen) inside the test tube. This limited the availability of oxygen supply that the yeasts required in order to grow aerobically.

The test tubes can be placed to ferment aerobically in a closed container such as BioFlo 3000. This kind of bio processing systems provide a wide range of options that enables the researcher to adjust a standard air flow which includes different options of certain proportions oxygen ggand air which can respond to oxygen-demanding yeasts or any other microorganism.

There was absence of some basic element sources in every yeast culture that are necessary for better fermentation conditions such nitrogen and phosphorus sources. Lack of such sources lead to relatively low cell growth comparing to the growth that could be achieved without the absence of such elements.

Bacto-peptone can be used as an organic nitrogen source. Yeast extract makes available many bio nutrients required for the fermentation of yeast cells. It also provides essential water soluble vitamins, amino acids, peptides and carbohydrates.

## Chapter 3: Data Collection and Processing

Calculation of cell concentration

In order to calculate the cell concentration for each factor, the comperative mean values, which are displayed above, were used. These mean values were applied to the following formula which enables to convert counted cells into cell concentration:

In the above formula, C is the viable cells/mL, N is the counted cells, D is the dilution factor and 103 is the haemocytometer correction factor.

An example with the application of the formula of cell concentration for the factor of temperature at 5oC and after 24 hours of fermentation is shown below:

In the case of 24 hours of fermentation at temperature at 5oC, the viable counted cells, N= 34. 25, the dilution factor, D= 1. In all experiments, when testing the different factors, the dilution factor is always one (D= 1).

Representation of calculated data of cell concentrations

Tables of cell conentration (cells/mL) for the differing temperature values:

Table with the initial population:

Temperatures() ±0. 5

Cells/mL (Chamber 1, Chamber 2) (counted cells)

Standard Deviation

Table with the 24 hours fermented population:

Temperatures() ±0. 5

Cells/mL (Chamber 1, Chamber 2) (counted cells)

Standard Deviation

Tables of cell conentration (cells/mL) for the differing pH levels:

Table with the initial population:

pH

Cells/mL (Chamber 1, Chamber 2) (counted cells)

Standard Deviation

Table with the 24 hours fermented population:

pH

Cells/mL (Chamber 1, Chamber 2) (counted cells)

Standard Deviation

Tables of cell conentration (cells/mL) for the differing glucose concentrations:

Table with the initial population:

Glucose 2% concentrations (mL)

Cells/mL (Chamber 1, Chamber 2) (counted cells)

Standard Deviation

Table with the 24 hours fermented population:

Glucose 2% concentrations (mL)

Cells/mL (Chamber 1, Chamber 2) (counted cells)

Standard Deviation

# Chapter 4: Analysis and Interpretation

## 4. 1 Graphs

The data that is used for the sketching of the graphs is shown in chapter 3, in “ Data Processing”, “ Representation of calculated data of cell concentrations”. The respective table values were used for each of the factors.

The software that was used for the sketching of the graphs is, Graph 4. 3 (Ivan Johansen, 2007).

effect of Temperature on S. cerevisiae population growth

The effect of pH on S. cerevisiae population growth

The effect of substrate Glucose concentration on S. cerevisiae population growth

## 4. 2 Interpretation

### Testing Hypothesis 1:

Comparing the different temperatures that the S. cerevisiae population left to grow, it can be seen based on both the cell concentration and the graph, that below 30oC the of the population grows rapidly as the temperature increases; the yeast population almost doubles when temperature increases from 5oC to 15oC and almost triples when temperature increases from 15oC to 30oC . Above 30oC the growth of the population is highly decreased; yeast population becomes almost 3. 5 times less when temperature increases from 30oC to 50oC and when temperature increases from 50oC to 60oC the population decreases very slightly. As a result, the highest S. cerevisiae

population growth is observed at 30oC. Consequently this should be the optimum temperature. Moreover, as temperature below the optimum point increases the population increases more from its initial value than it does at temperatures above the optimum point. Overall the hypothesis confirmed.

**Testing Hypothesis 2:**

Evaluating the yeast population growth at the different pH levels, it can be seen that the increase of population above and below the value of pH 6 is almost the same. The fact that at pH 6 it is observed the highest population growth implies that this is the optimum pH level. The lowest growth is observed at pH 3 and pH 8. In these specific pH levels the growth is slightly higher at pH 8 (population increases approximately 1. 7 times) than it is at pH 3 (population increases approximately 1. 3 times). The growth is higher in pH 8 as it is closer to the optimum pH. At pH 4 the increase in population is almost the same as it is at pH 8. Both pH 4 and pH 8 differ by 2 pH levels from the optimum level but the yeast population at pH 4 increases approximately 1. 982 times where at pH 8 the population increases 1. 7 times. This shows that S. cerevisiae operates better at acidic conditions. Overall the hypothesis is confirmed.

**Testing Hypothesis 3:**

Analysing the growth of S. cerevisiae at different glucose concentrations and for 24 hours of fermentation, the results obtained show that in the absence of glucose from the culture the yeast population didn’t increase at all. The only increase that was observed from its initial population was 1. 091. 1 times, meaning that this 0. 1 increase may have occurred due to the

capacity of energy within the yeast cells. At 1% glucose concentration it was observed sufficient growth. The yeast population almost doubled from its initial value (increased approximately by 1. 8 times). In higher glucose concentration the yeast cells population respond greater and as a result a higher population growth was observed. The initial population increased 3. 9 times, meaning that almost quadrupled. In even higher glucose concentrations the population increased highly again but not enough so to be able to say that at 24 hours of fermentation S. cerevisiae requires more energy to reach the maximum replication capacity. The population increased 3. 954. 00 times, almost the same of that of 2% concentration. Moreover, based on the graph plotted for glucose concentrations, it can be seen that after 2% glucose concentration the yeast population reaches plateau without any further increase. So the limiting growth glucose concentration is at 2%. Overall the hypothesis is confirmed.