First name ……. Date …….
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Exercise B40
Effect of temperature on the rate of cellular respiration in yeast.

Table 1. Temperature dependence of oxygen consumption rate.

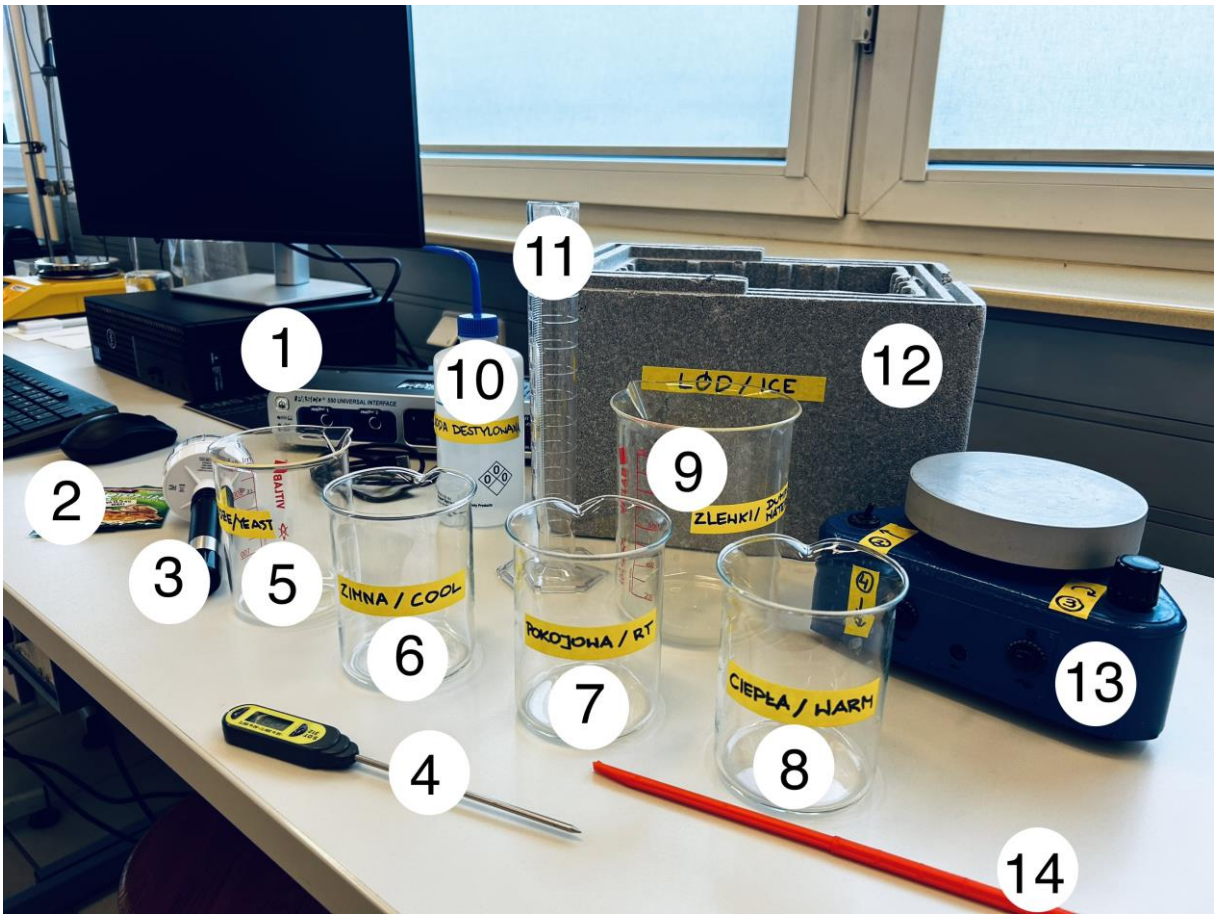
Conditions	**Water temperature (^{o}C)**	**Initial oxygen concentration (mg/l)**	**Final oxygen concentration (mg/l)**	**Time (s)**	**Change in oxygen concentration (mg/l)**	**Intensity of cellular respiration (μg/(l*s)**
Water at room temperature						
Warm water						
Cold water						

Table 2. Changes in oxygen concentration over time.

Time	0s	20s	40s	60s	80s	100s	120s
	Oxygen concentration (ml/l)						
Water at room temperature							
Warm water							
Cold water							

Workstation (description to the photo below):

1. Computer with an interface
2. 7g instant yeast
3. PASCO dissolved oxygen sensor
4. Electronic thermometer
5. 400 ml beaker for yeast suspension
6. 400 ml beaker for cold water
7. 400 ml beaker for water at room temperature
8. 400 ml beaker for warm water
9. 1-litre beaker for dirty water
10. Wash bottle
11. Measuring cylinder
12. Polystyrene ice container
13. Heating plate
14. Mixing rod



Introduction

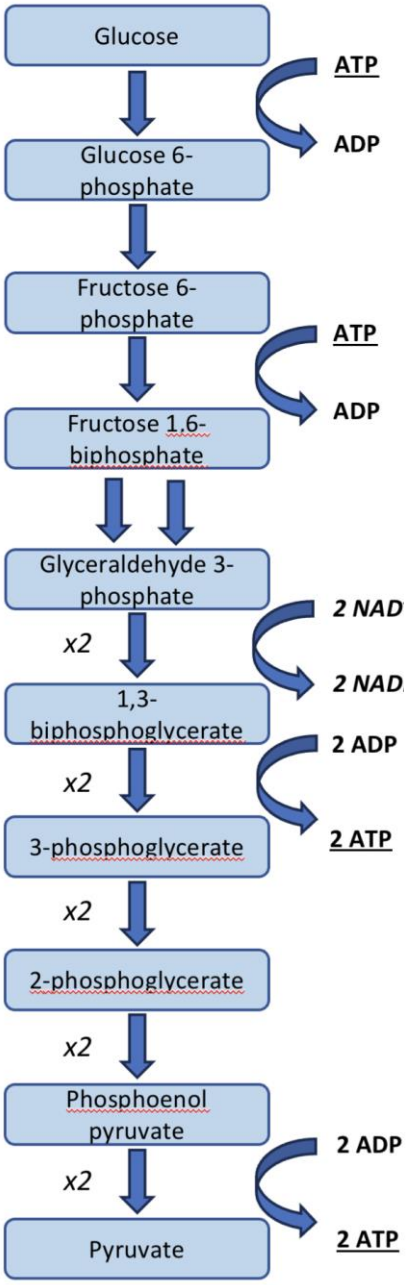
Every living organism needs biologically useful energy (in the form of ATP) to function and grow properly. This energy can be produced with oxygen in mitochondria or without oxygen e.g., in the process of fermentation. Living organisms can use a variety of organic compounds to produce energy, the most important of which are carbohydrates (the most readily available source of energy), fats (the main spare material in animals) and proteins (used last).
We can divide intracellular respiration into several stages:

1. Glycolysis, which takes place in the cytoplasm. During this process, glucose is oxidised to pyruvate (Figure 1). The energy gain is 2 molecules of ATP. The ATP formed during glycolysis is the result of the transfer of a phosphate residue from a substrate to ADP and is called substrate phosphorylation. Glycolysis can occur under anaerobic conditions.
2. Link reaction occurring in the mitochondrial matrix. During the link reaction, oxidative decarboxylation of pyruvate occurs. Pyruvate is degraded and combines with coenzyme A to form acetyl-CoA. Carbon dioxide is released during this transformation.
3. The Krebs cycle, otherwise known as the citric acid cycle or tricarboxylic acid cycle, occurs in the mitochondrial matrix. During a series of transformations in this process, NADH and $FADH_2$ necessary for oxidative phosphorylation are produced. During the Krebs cycle, substrate phosphorylation also takes place, during which GTP is produced. The acetyl group from acetyl-CoA is degraded to carbon dioxide.
4. Oxidative phosphorylation, occurring in the inner mitochondrial membrane, in which NADH and $FADH_2$ are used to synthesise ATP. Hydrogen cations from these compounds form a proton gradient across the inner mitochondrial membrane. ATP is formed by the movement of protons according to their concentration gradient through ATP synthase. At the same time, electrons are transferred along the chain. The final hydrogen acceptor is oxygen.

Dissolved oxygen sensor

Dissolved oxygen concentration measurements, carried out over time, are used to assess the rate of cellular respiration. From changes in the oxygen concentration, the rate of oxygen consumption can be determined and, consequently, the rate of cellular respiration can be assessed. Dissolved oxygen sensors are used to assess changes in oxygen concentration. These sensors can be divided into two types: electrochemical and optical.
An optical dissolved oxygen sensor will be used during this exercise. Optical dissolved oxygen sensors (called luminescent sensors) measure dissolved oxygen concentration based on the extinction of luminescence in the presence of oxygen. These sensors are made up of light-emitting diodes, a luminescent dye and a photodetector. The measurement method is based on the physical phenomenon of luminescence. Luminescence is the emission of light due to the action of a light pulse. When the sensor is operating, the light pulse is emitted by an LED, which is placed in the sensor. Due to the action of the light pulse, the phosphor emits red light. The intensity of this emission depends on the dissolved oxygen concentration. The red light emission time is calculated to determine the oxygen concentration.

The aim of the exercise is to compare the intensity of cellular respiration of Saccharomyces cerevisiae under different temperature conditions using an optical dissolved oxygen sensor.



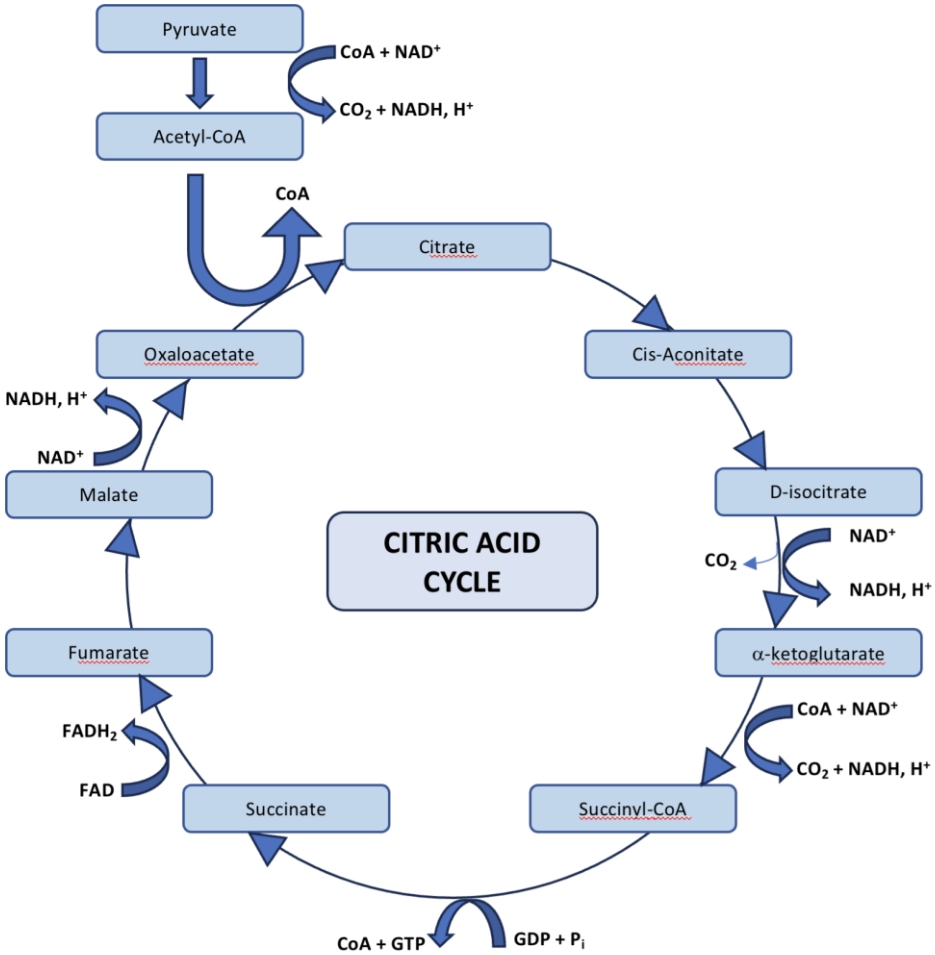


Figure 2: The citric acid cycle. Pyruvate derived from glycolysis undergoes decarboxylation to acetyl-CoA. This in turn enters the citric acid cycle. In the subsequent steps of this cycle, CO_2 and high-energy compounds in the form of NADH, $FADH_2$ and GTP are produced from organic compounds.

Figure 1: Schematic of glycolysis. In the first step, glucose is phosphorylated by hexokinase consuming 1 molecule of ATP (adenosine triphosphate). The resulting glucose-6-phosphate molecule is converted to fructose-6-phosphate by hexose-phosphate isomerase and then phosphorylated to fructose-1,6-bisphosphate using another molecule of ATP. The resulting fructose-1,6-bisphosphate is broken down into two molecules of 3-phosphoglyceraldehyde. The 3-phosphoglyceraldehyde is phosphorylated and oxidised to 1,3-bisphosphoglycerate with the formation of an NADH molecule. Conversion to 3-phosphoglycerate results in the production of an ATP molecule. Another ATP molecule is produced at the final stage of glycolysis-the formation of pyruvate.

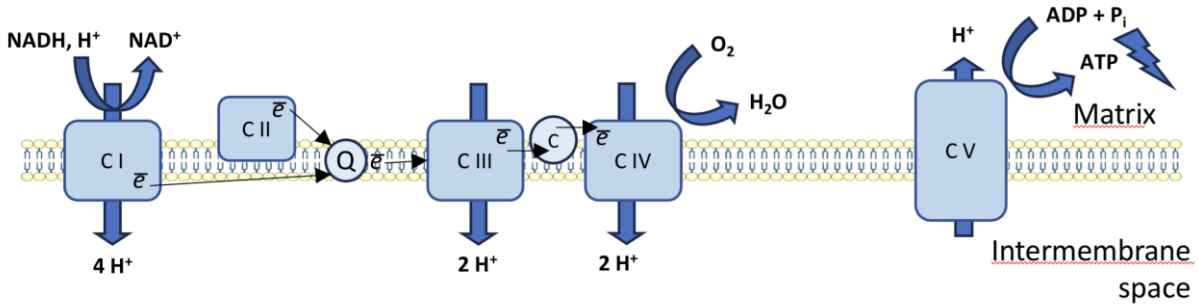


Figure 3: Oxidative phosphorylation. The reduced nucleotides NADH and $FADH_2$ formed in the Krebs cycle can be oxidised on enzyme complexes that form part of the respiratory chain located in the inner mitochondrial membrane. Oxidative phosphorylation results in an electrochemical gradient, which serves to synthesise ATP. As a result of the oxidation of NADH and $FADH_2$, protons from the mitochondrial matrix are transferred to the intermembrane space. The energy stored in the form of a proton concentration gradient and potential difference, referred to as the electrochemical potential, is used to produce ATP.

Complex I is called NADH dehydrogenase. During the oxidation of NADH, protons from the mitochondrial matrix are transferred to the intermembrane space. The transfer of two electrons from NADH to ubiquinone is accompanied by the transfer of four protons from the matrix to the intermembrane space.

Complex II is one of the Krebs cycle enzymes, succinate dehydrogenase, which contains several iron-sulphur centres and a flavinadenine dinucleotide. Electrons from reduced FADH2, as in complex I, are transferred via the iron-sulphur centres. Complex II is not a trans-membrane protein and does not have the ability to transfer protons across the inner membrane.

The ubiquinone reduced on complex I or II moves across the mitochondrial membrane to complex III of the respiratory chain called complex bc1. A Q-cycle occurs at complex III, whereby additional protons are transferred from the matrix to the intermembrane space. Electrons from the reduced ubiquinone are transferred to cytochrome c, a small hydrophilic protein located on the intermembrane space side, which transfers electrons to complex IV after reduction at complex III.

Complex IV is a cytochrome oxidase that transfers electrons from cytochrome c and transfers them to the O_2 molecule. Complex IV transfers protons to the intermembrane space from the mitochondrial matrix. At the same time, an H_2O molecule is formed.

As a result of electron transfer between complexes I-IV, H^+ ions are transferred from the mitochondrial matrix to the intermembrane space. The energy stored in this form is used by complex V (ATP synthase). ATP synthase consists of an F0 subunit which is an ion channel and an F_1 subunit located on the matrix side that attaches inorganic phosphate to the ADP molecule, using the energy of the electrochemical potential. The generation of one molecule of ATP requires the transfer of 3.33 protons into the matrix.

Performance of the task:

Equipment and reagents setup:

1. Turn on the power to the bench by turning the red knob under the table to the right (it should pop out) and then turning the key and releasing it. A green LED should light up on the bench.
2. Switch on your computer and start the data acquisition programme "B40_Respiration in yeast" on your desktop.
3. Switch on the interface next to the computer (button should light up blue).
4. Turn on the dissolved oxygen sensor using the button on the white part of the sensor.
5. Make sure that the DO_2 concentration measurement from the dissolved oxygen sensor is marked along with the temperature measurement.
6. Label the four 400ml beakers: 'RT' (room temperature), 'Cool', 'Warm' and 'Yeast'.
7. Add 200 ml of distilled water at room temperature to each 400 ml beaker.
8. Fill the polystyrene container to 1/3 of its volume with ice. Place the "Cool" beaker in the so that all 200 ml of distilled water inside the cool beaker is in contact with the ice, but do not allow the ice water from outside to enter the beaker.
9. Place the 'Warm' beaker on the hotplate. Turn the hotplate on to the low setting. To turn on the hotplate switch the switches as shown on the hotplate housing. Insert the magnetic stirrer and insert the electronic thermometer. Wait until the water has heated up to approximately 35°C.
10. Add 7g of instant dry yeast to the "Yeast" beaker and stir to form a homogeneous suspension.
11. Remove the rubber cap from the dissolved oxygen sensor (DO_2). Avoid touching the bottom of the sensor.

Performing measurements:

1. Place the tip of the electronic thermometer in water in a beaker at room temperature. When the reading stabilises, record the temperature of the water in Table 1. Remove the thermometer, rinse it over a large beaker and set it aside.
2. Hold the DO_2 probe in a beaker of room temperature water as shown in Figure 4. Do not allow the probe to come into contact with the beaker and keep the metal part at the end of the probe fully submerged. Do not allow the sensor housing to become wet.
3. Gently stir the water with the DO_2 probe for 30 seconds.
4. Stir the activated yeast suspension with the rod. Measure 50 ml of the suspension with a measuring cylinder and pour it into a beaker at room temperature. Stir gently (the DO_2 sensor should remain in the beaker at all times).
5. On the computer, in 'Room temperature' tab, select START to start data collection. Record the initial DO_2 concentration in Table 1. Continue mixing and recording data for 2 minutes (120 seconds).
Stop data collection with the STOP button. Record the initial and final DO_2 concentration and exact elapsed time in Table 1.
7. Rinse the DO_2 sensor over a large beaker and set it aside.
8. From the table on the computer, select 7 oxygen concentration readings, every 20s, and record them in Table 2.
9. Repeat steps 1-8 with the 'Warm' beaker, switching the tab in the data collection software with the arrow to the 'Warm' tab. Leave the beaker on the hotplate during the procedure.
10. Turn off the hotplate immediately after measurements, following the instructions on the hotplate housing in reverse order.
11. Repeat steps 1-8 with the "Cold" beaker, switching the tab in the data collection software with the arrow to the "Cool" tab. Leave the beaker on ice while you perform the procedure.
12 Put the rubber cap on the DO_2 probe.

Figure 4. Oxygen sensor with metal part submerged in water.

13. Use the following equation to calculate the DO_2 change for each run; record the result in Table 1.

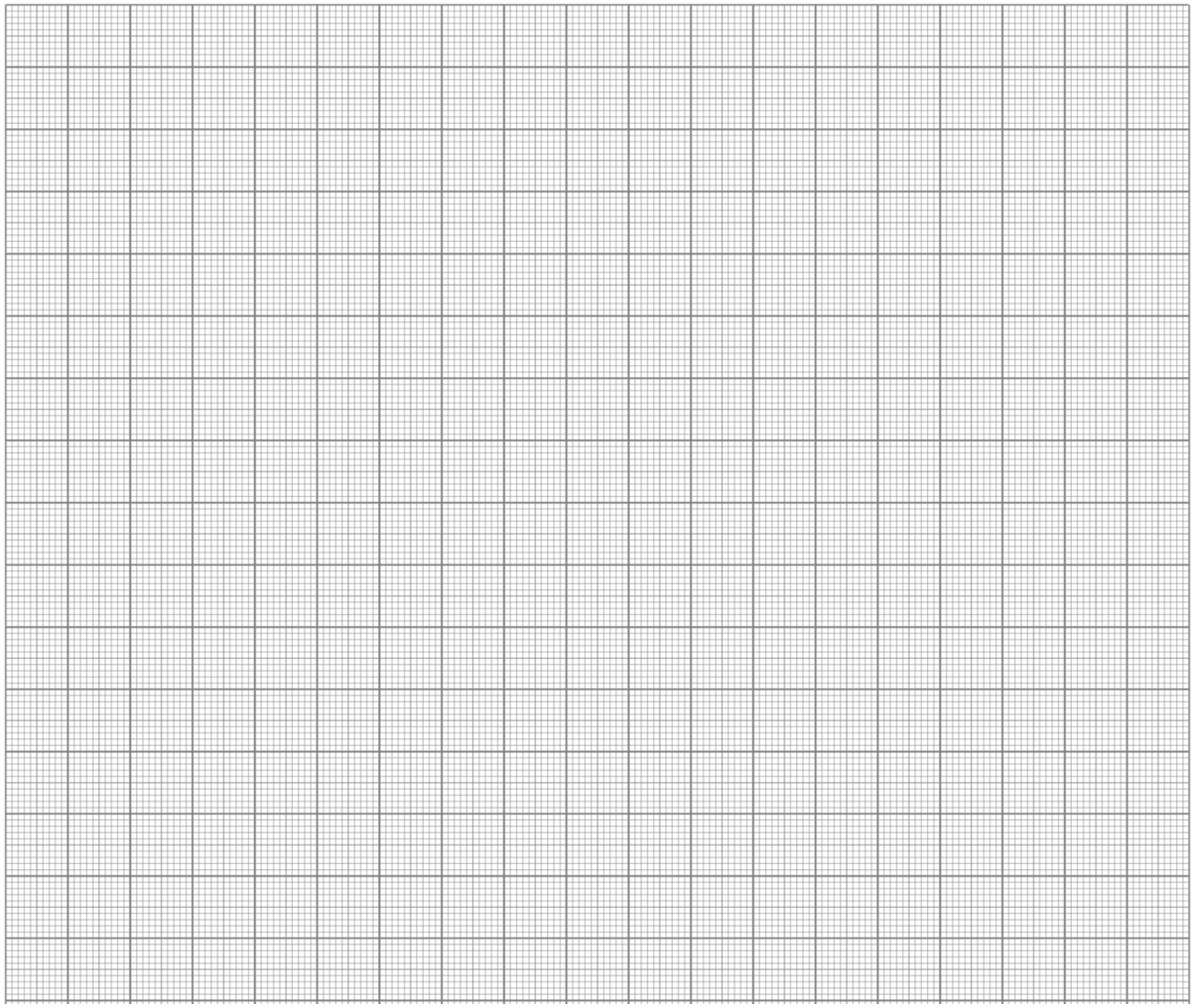
Change in DO_2 = Final DO_2 - Initial DO_2

14. Use the following equation to calculate the rate of oxygen consumption by the yeast and record the result in Table 1. Remember to convert units!

Rate of DO_2 consumption = Change DO_2 ÷ Time

Data analysis:

1. To analyse the data, present the data from Table 2 on a graph of c(t) using the millimetre paper below. Remember to select the scale accordingly.
2. Plot the error of the oxygen concentration, which is 0.5mg/ml.
3. Discuss the differences between the graphs under different temperature conditions in the conclusion part of your report.



Additional questions:

1. In which temperature did the DO_2 level decrease most rapidly? What does this indicate about the environmental needs of yeast? Support your answer with data.

2. Why is oxygen consumption a good measure of respiration rate in yeast? Include a description of how oxygen molecules are consumed and recombined during cellular respiration.

3. If oxygen is not present, respiration can still occur. What is that process called? What additional end products are produced?

4. Explain the difference in the amount of energy produced in aerobic versus anaerobic respiration.

Refereces

Alberts B., Hopkin K, Johnson A.D., Morgan D., Raff M., Roberts K., Walter P. „Essential Cell Biology", WW Norton&Co 2019
Urry L.A., Cain M.L., Wasserman S.A. Campbell Biology", Pearson Education Limited 2020
pl.wikipedia.org