EXAMINATION RULES

1. All participants must be present at the front of the examination room ten minutes before the examination starts.

2. Participants are not allowed to bring any tools except his/her personal medicine or any personal medical equipment.

3. Each participant has to sit according to his or her designated desk.

4. Before the examination starts, each participant has to check the stationary and tools (pen, eraser, ruler, sharpener, pencil, calculator) provided by the organizer.

5. Each participant has to check the question and answer sheets as well as the experimental set up provided. Raise your hand, if you find any missing sheets or incomplete experimental set up. The supervisor will provide you with what is lacking. Start after the bell.

6. The participant must write down his/her code and country (in Latin characters) on each of the answer sheet. The answer must be written on one side of the answer sheet.

7. During the examination, participants are not allowed to leave the examination room except for emergency case and for that the examination supervisor will accompany them.

8. The participants are not allowed to bother other participant and disturb the examination. In case any assistance is needed, a participant may raise his/her hand and the nearest supervisor will come to help.

9. There will be no question or discussion about the examination problems or any damage of equipment due to negligence or careless handling. The participant must stay at their desk until the time allocated for the examination is over, although he/she has finished the examination earlier or does not want to continue working.

10. At the end of the examination time there will be a signal (the ringing of a bell). You are not allowed to write anything on the answer sheets, after the allocated time is over. All participants must leave the room quietly. **The question and answer sheets must be put neatly on your desk.**

EXPERIMENTAL EXAMINATION

December 09, 2006

Read carefully the following instructions:

1. The time available is 4.0 hours.
2. **Be sure that** your team has **a complete set of the experimental examination** (3 copies) **and the answer sheets** (4 copies). Only one copy should be submitted from the team for marking.
3. A complete set of the experimental and apparatus material are provided on your desk as described in the experimental instructions
4. **Be sure that the kit works properly. When you find the kit does not work properly, raise your hand for replacement. No complaint will be responded to after the examination has been started except for the occurrence of certain accident.**
5. Write down your code, country and signatures of each of the team members on the final answer sheet, using the pen provided. Your answers must be written down on one side of the answer sheet provided.
6. **After completing the examination, put the answer sheets as well as the problem sheets on your desk.**
7. **Discard all chemical solutions after completing the experimental tasks.**

EXPERIMENTAL EXAMINATION

December 9, 2006

Maximum points for problem is 40

A. Introduction

The following experiment deals with the phenomena of respiration, especially fermentation （發酵）, in living organisms. The respiration phenomenon that usually occurs in mitochondria of eukaryotes is characterized by the consumption of organic molecules to generate energy for all biological processes in the cell. In the respiration process, oxygen and organic compounds are consumed and carbon-containing molecules are released into the environment. In the unicellular organism, *Saccharomyces cerevisiae* (bakers yeast 酵母菌), another form of energy-producing pathway occurs. This is the fermentation process, where oxygen is not an available component. In the fermentation process the same organic substances can be metabolized, but less energy is produced.

In this experiment, a simple set of laboratory tubes (experimental vessels) is presented, as schematically shown in Figure 1, 2 and 3. These will be used to:

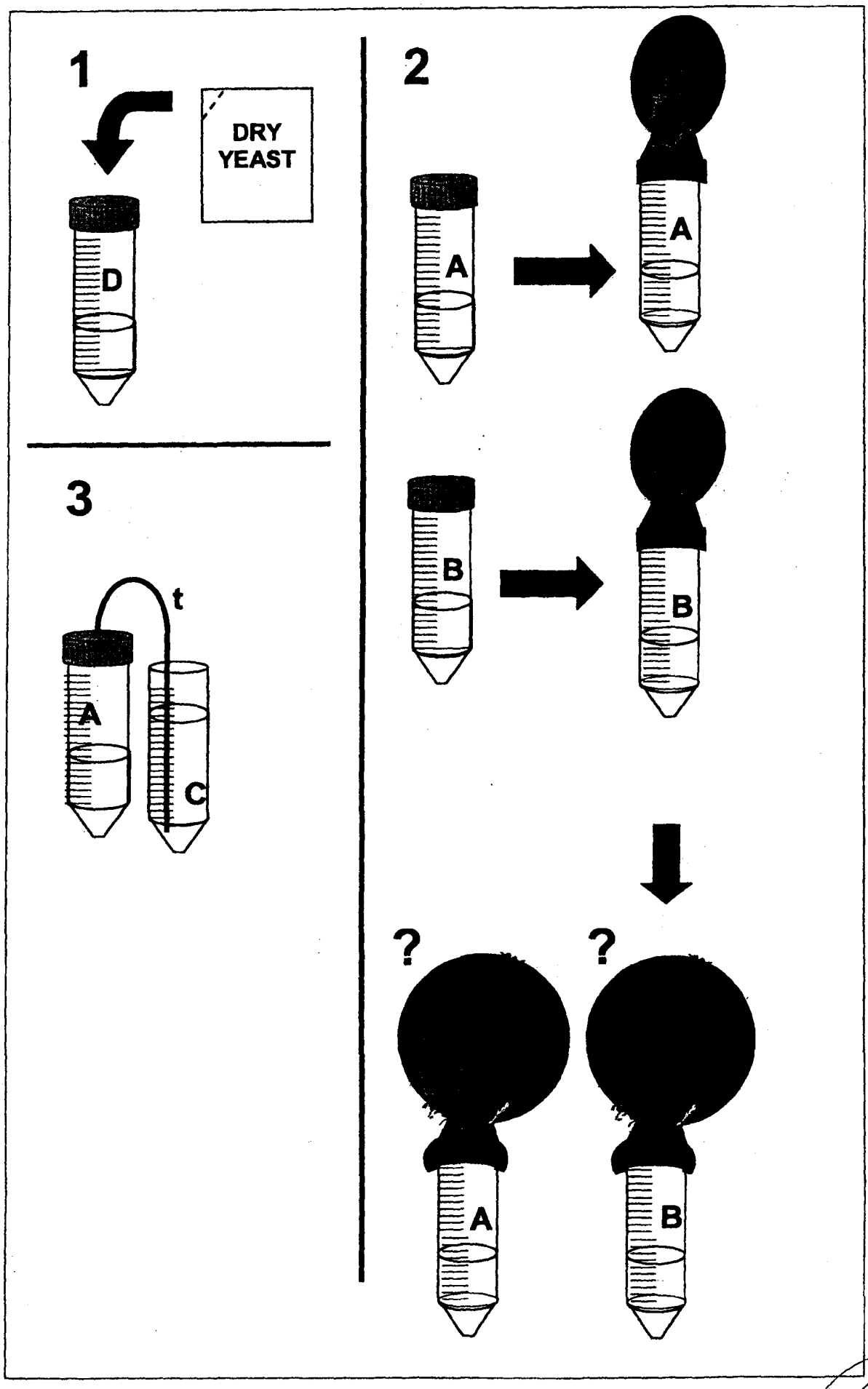
a) Evaluate the respiration process in bakers yeast (*Saccharomyces cerevisiae*)
b) Evaluate the influence of the organic substance available to the yeast cells.
c) Estimate the speed of the reaction.
d) Identify the effect of the gas produced on the pH of the media.

The experimental kit contains the following materials:

1- Five graduated（有刻度的）plastic laboratory tubes with covering cap and filled with the required solutions, marked A to D.
2- Four party balloons.
3- An envelope containing dry yeast cells.
4- A piece of string. (15 cm)
5- A stand for the experimental tubes.

The experiment is divided in two parts:

1 – Tube A with a tube-fitted cap and a tube containing a color solution of Phenolphthalein (Tube C).
2 – Two tubes (A and B) containing glucose solution (A) and starch solution (B). Party balloons, measuring string and ruler.


1
DRY YEAST
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2
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3
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B. Objectives

B.1 General Objective

To demonstrate experimentally the respiration process in yeast.

B.2 Specific Objectives

a. To evaluate the effect of different substrates on the rate of the reaction in bakers yeast (*Saccharomyces cerevisiae*)

b. To estimate the speed of the reaction.

c. To characterize the effect of the gas produced in tube A by observing the color change of the indicator solution in tube C.

C. Apparatus and Materials

C.1 *Materials :*

1.	Tube A	2 Tubes (20 mL of glucose)
2.	Tube B	1 Tube (20 mL of starch)
3.	Tube C	1 Tube (30 mL of Phenolphthalein)
4.	Tube D	1 Tube (30 mL of water)

D. Experiment

D.1 **General Instructions**

In the following experiments, you have to measure the formation of gas and its effect on the pH of the indicator solution of Phenolphthalein. Determine the volume of gas produced by yeast cells from two different carbon sources, glucose and starch.

Read the instructions carefully before doing the experiments.
In each experiment, there will be questions related to the particular experiment.
Answer all questions on the answer sheets provided.

D.2 **Preparation steps (Figure 1).**

The following preparation steps should be carried out first, before doing any experiment.

1. Verify if your experimental kit has all the material listed.
2. Open the envelope containing dry yeast cells. Add the equivalent to 10 mL of yeast cells into tube D, using the calibrations on the tube, to make the final level up to 40 mL.
3. Shake the mixture vigorously （劇烈地） in Tube D to disperse the yeast cells. **Take note of the final volume.**
4. Do not let the solution in Tube D stand for very long. Go quickly to the next experiments (1 and 2) simultaneously （同時地）. Do not close this tube tightly.

Experiment-1 (Figure 2): Effects of carbon source on the respiration of yeast.

I-1 Add 10 mL of yeast solution from Tube D into one Tube A and also 10 mL into Tube B. Close Tube A and Tube B with the party balloon (as shown in figure 2).
Make sure the balloons are as empty as possible when covering the tubes. You have extra balloons if one tears at this stage. Make sure the balloons are securely fitted.

I-2 Let the tubes (with the fitted balloons) stand for 1 hour. At 10 minutes time intervals, gently mix the solution of the tubes. (每十分鐘輕輕搖動溶液一次)

I-3 After 1 hour measure the diameter of the balloon that is filled with gas.

I-4 Considering the balloon as a sphere, calculate its volume. Remember that it is not required to consider the resistance of the plastic balloon.

Hint: You can tie a knot in the balloon to help it become a sphere, so far that you do not let gas escape or force the balloon excessively.

Experiment-2 (Figure 3): Effects of gas formation on the pH indicator solution of Phenolphthalein.

II-1 Add the remaining 10 mL of yeast solution into the Tube A that has its cap fitted with plastic tubing and close as shown in figure 3).

II-2 Introduce the other end of the tubing into Tube C and from time to time gently stir Tube A.

II-3 At times 5min, 15min, 25min, 35min and 45min counting from the start of the experiment record the time that each bubble is released during a period of 1-min in the table on the answer sheet for question 18 (e.g. release of the first bubble after 10s, second after 18s ...) (在實驗開始後第 5, 15, 25, 35, 及 45 分鐘的那一刻後的一分鐘內，紀錄汽泡出現的時間於答題紙第 18 條的表上。例如：第一個汽泡在第 10 秒出現，第二個汽泡在第 18 秒出現) The reaction may start very slowly. Please still continue working.

II-4 After 30 minutes also record the color of the solution C for question 18.

II-5 During the next 30 minutes, pay attention to the solution color and take note of the moment that it completely looses its color.

E. ***A. Questions – Please write all your answers on the answer sheet!***

In most questions, you are provided with four possible answers. Read the question very carefully and then choose one correct answer by crossing one of the answers provided on the answer sheet. It should be noted that there is only one correct answer for each question. Write your calculations and data in the answer sheet.

1. Write the balanced chemical equation involved in the use of glucose by yeast under anaerobic conditions (缺氧呼吸). (1pt)

2. Calculate the amount of glucose consumed by yeast (in grams) if 88 grams of carbon dioxide is released in this experiment. (2pt)

3. Which biological process took place in tube A during the first 5 minutes? (1pt)
   A. fermentation (anaerobic respiration)（缺氧呼吸）
   B. aerobic respiration（帶氧呼吸）
   C. Photosynthesis（光合作用）
   D. Chemosynthesis（化合作用）

4. Considering the process in question 3, which liquid or gaseous product is produced? (1pt)
   A. O_2
   B. CO_2
   C. CH_4
   D. C_2H_5OH

5. Which biological process will take place in tube A after oxygen is exhausted and excess of glucose is still present? (1pt)
   A. fermentation (anaerobic respiration)
   B. aerobic respiration
   C. photosynthesis
   D. chemosynthesis

6. Considering the process in question 5, which metabolite is produced? (1pt)
   A. O_2
   B. CO
   C. CH_4
   D. C_2H_5OH

7. What happens to the color of the phenolphthalein solution after bubbling the gas from tube A in experiment 2? (1pt)
   A. stays pink
   B. turns yellow
   C. turns green
   D. becomes colorless

8. What is the reason for your observation in question 7? (1.5pt)
   A. The product of the process in tube A makes the solution basic, and at basic pH phenophthalein changes color.
   B. The product of the process in tube A makes the solution acidic, and at acidic pH phenophthalein changes color.

C. The product of the process in tube A leads to evaporation of phenolphthalein, therefore the color changes.

D. The product of the process in tube A causes precipitation of phenolphthalein, so the color of the solution changes.

9. Compare the size of the balloons from tube A and B and choose the correct answer. (1pt)

A. Both balloons are almost empty.

B. Balloon A is larger than balloon B.

C. Balloon B is larger than balloon A.

D. Both balloons A and B are filled and have about the same size.

10. What is the explanation for your observation in question 9? Tube A contained glucose and tube B contained starch. (1.5pt)

A. Yeast uses both glucose and starch as a carbon source, but glucose is more efficient.

B. Yeast can not use starch as a carbon source.

C. Starch is used only in fermentation and not in respiration.

D. Starch is toxic to yeast cells.

11. What would happen, if the tubes in experiment 1 were not shaken regularly (step I-2)? (1.5pt)

A. The yeast would not be disturbed in its metabolism （代謝作用） therefore the reaction would be too fast to measure it.

B. The process would be slower because the liquid would not be mixed to distribute substrates and metabolites（代謝物）

C. The process would be slower because the liquid would not be aerated （給予氧氣）, enriched with oxygen from the volume above the solution.

D. both B and C are correct

12. Consider the following theoretical changes in the setup of the experiment:
Tube A – kept at room temperature as in the experiment that you have performed
Tube A2 – kept warm in the hands during the whole incubation period
Tube A3 – kept on ice during the whole incubation period
Tube A4 – was boiled for 5 minutes before start of the experiment and kept at room temperature during the incubation period
Which option for the comparison of the balloon sizes would you expect? (1.5pt)

A. A3 < A < A2 < A4

B. A2 < A < A3 < A4

C. A4 < A3 < A < A2

D. A3 < A4 < A < A2

13. When baking bread, it is common to use yeast together with flour, sugar and salt to make the dough 麵粉團. Why does the bread dough rise? (1.5pt).

A. Because the yeast in the dough uses the glucose to produce CO_2 which is trapped in the dough as bubbles of gas, causing the bread to rise.

B. Because the yeast in the dough uses the flour (starch) to produce CO_2 that is trapped in the dough as bubbles of gas, causing the bread to rise.

C. Because the yeast in the dough uses both the flour (starch) and the glucose to produce CO_2 that is trapped in the dough as bubbles of gas, causing the bread to rise.

D. Because the flour (starch) and the glucose react with the salt added, to produce CO_2, that is trapped in the dough as bubbles of gas causing the bread to rise.

14. The 10 mL of yeast are equivalent to 5.0 grams that were added to the 30.0 mL of water in Tube D. What is the concentration (grams/mL) of yeast in tube D? (2pt).

15. Then if you used 10.0 mL of this yeast solution from tube D, from the problem number 14, and added it to tube A. What is the concentration (grams/mL) of yeast in tube A? (2pt).

16. Consider the balloon as a perfect sphere. Calculate the volume of gas trapped in the balloon of tube A at the end of experiment 1. The volume of a sphere of radius r is given by $V = 4/3\ \pi\ r^3$. (2pt)

17. Using the value for the final volume of the balloon, calculate the rate of gas formation in tube A of experiment 1. (2pt).

18. From the data collected in Experiment 2 plot a graph for the three most significant time intervals of 1 minute （從實驗 2 中選三組最有代表性的數據來各畫一幅圖） on the answer sheet for question 18. Plot bubble release time (vertically) versus bubble number (horizontally). (10.5pt)

19. Determine the slope of each of the graphs prepared in question 18. Use these results to calculate the rate of gas production at that respective time. (Do not estimate the errors.) The inner diameter of the plastic tube is 3 mm. (3pt)

20. What is the reason for the difference between the rates of gas production calculated in experiments 1 and 2? (1pt)

A Change of pressure in the balloon during the experiment

B Change of temperature during experiment

C Presence of Phenolphthalein in the second experiment

D There was no difference between the calculated rates of gas production.

21. Which value is more likely to correctly represent the gas production rate at the end of the experiment? (1pt)

A Value calculated in experiment 1

B Value calculated in experiment 2

C Both values are equally correct.

D Both values are not suitable estimates.