Yeast Cell Wall/Osmosis experiment –

In this experiment, students will remove the cell wall from a yeast cell. Students will observe that without a cell wall, a yeast cell cannot maintain homeostasis and dies.

Using an enzyme, students will remove the cell walls from yeast cells while leaving the cell membranes intact. Under the microscope, they will not see a difference between yeast cells that lack a cell wall and those that have a cell wall. However, a difference becomes apparent when water is added. The yeast cells with intact cell walls are unaffected. The yeast cells lacking cell walls explode.

What happened? In unaffected cells, cell walls block the entry of water. The cell membrane, however, is water-permeable. When no cell wall is present, water rushes in, exceeding the capacity of the cell membrane. These cells explode.

From this exercise, students will learn:

(1) Yeast cells, like all members of Kingdom Fungi, have a cell wall.

(2) The cell wall and cell membrane are structurally and functionally distinct.

(3) The cell wall is important for maintenance of a fungal cell's homeostasis and structure.

(4) Osmosis occurs when water moves from an area of higher concentration to one of lower concentration through a semi-permeable membrane.

(5) Basic laboratory and microscope techniques:

pipetting	centrifuging
preparing a slide	focusing a microscope

Student questions:

1. What kind of molecule is zymolyase? What does it do?

2. Describe what you saw after you added water to the control cells. What about the zymolyased cells?

3. Explain your observations from question 2.

Protocol to Remove the Cell Wall
of the Budding/Bakers Yeast (*Saccaromyces cerevisiae*)

1 pkg bakers yeast	10 mL, 200 mM Hepes	10 mL spheroplasting buffer
100 mL YPD	2 glass pipettes	1 mL 5mg/ml zymolyase solution
30 ^{0}C incubator	1mL, 1M DTT	

Using sterile technique, grow a 1 mL yeast starter culture (1 colony, overnight, in 1mL YPD at 30 ^{0}C with shaking at 300 rpm). Add this culture to 90 mL YPD. Grow at 30 ^{0}C, 300 rpm to an OD of between 1.0 and 1.3 (6 to 12 hours). At this stage, culture may be refrigerated for several days before use.

When ready to continue, spin cells out of solution (2100 rpm for 3'). Decant liquid. Resuspend the cells in 5 mL, 200 mM Hepes, pH 7.5 using a glass pipette. Add 500 uL of 1M DTT (final concentration 100 mM). Gently invert this mixture once every 30 seconds for a total of two minutes.

As before, pellet and decant. Resupend pellet in 5 mL spheroplasting buffer using a glass pipette. Split the culture in half by pouring approx. equal amounts into two, 15 ml conicals. Label one conical "zymolyase" and the other "control." To the "zymolyase" conical, add 100 uL zymolyase solution (200 ug/ml final). To the other, add 100 uL spheroplasting buffer.

Incubate with GENTLE shaking for 30' at 30 ^{0}C. Invert the tube after the first 2 minutes, and then, every 5 minutes. Cell walls have now been removed. If necessary, this may be refrigerated for a couple of days.

Viewing the cells

1) Pipette about 2 uL cells onto a slide. Use one slide for the "zymolyase" cells and one for the "control" cells. Do not use a coverslip.
2) Focus on the cells under the microscope.
3) Once cells are in focus, add 100 uL distilled water directly to the cells.
4) Refocus. Observed cells for about 2 minutes.

Sphereoplasting buffer:

0.4 M sorbitol	0.73 g
0.4 M KCl	0.298 g
40 mM KHPO4	400 uL of 1 M KHPO4
0.5 mM MgCl2	5 uL 1 M MgCl2
	to 10 mL with dH20

Zymolyase Solution:
Add 5 mg zymolyase to 1 mL spheroplasting buffer.