

Methods of Bacterial Cell Lysis: We need to lyse the bacterial cell for isolation and purification of the recombinant protein. Basics for the same have been discussed below.

Cell lysis is the **disruption of the cellular boundary**. In the laboratory this is usually done for the purpose of exposing the contents of the cell to purify and study them.

When formulating a cell lysis protocol following points must be considered:

- the contents of the cell should **remain unharmed** in the process.
- a buffer that can mimic the environment of the cell.

In general, there are two different methods to lyse bacterial cells.

1. Using lysozyme: It refers to use of enzyme lysozyme for bacterial cell lysis. The enzyme destroys the bacterial cell wall while freeze thaw helps to break the cell membrane. During freezing ice crystals are formed on the membrane which help break the membrane while during thawing leads to swelling and consequent lysis of the cells. The outline protocol is discussed below.

- Pellet the cells in culture by centrifuge.
- Re-suspend the cells in a buffer with pH around 8 as lysozyme is most active around pH 8. The buffer must have EDTA as one of the components.
- Add lysozyme in the re-suspended cells.
- Add PMSF in the mix. PMSF = Phenyl Methyl Sulphonyl Fluoride and it is serine protease inhibitor. This is needed to inactivate the proteases present in the lysate otherwise they might degrade the protein of interest.
- Incubate while shaking at 37°C for 30 minutes.
- Freeze using liquid Nitrogen and then thaw again at 37°C for 15 minutes.
- The lysate will be very viscous now due to presence of DNA.
- Add DNase and MgSO_4 and incubate while shaking for 15-30 minutes.
- Centrifuge the treated lysate now and collect the supernatant which contains proteins.

2. Using sonication: It refers to use of sound energy to break the cell wall and cell membrane so that contents of the bacterial cell can be isolated. It is also called as ultrasonication because we use sound frequencies of > 20kHz. The outline protocol is discussed below:

- Pellet the cells in culture by centrifuge.
- Resuspend them in a suitable buffer which also contains PMSF, a serine protease inhibitor.
- Place the sample in ice bath so that it becomes chilled. This process is very essential because during sonication heat is produced which could lead to damage of proteins. Chilling helps to dissipate the heat without harming the cell contents.
- Place the solution along with ice bath for sonication. The probe must be completely submerged in the sample but should not touch the bottom of the sample container.
- Set the frequency in the machine which is generally in the range of 20 – 50 kHz. Also set the duration of sonication which is generally 10-30 seconds and with this set the interval between two sonication events which is generally 30 seconds. This interval is set to ensure that heat generated during sonication has been dissipated before the next cycle and sample remains cool.

- After this we set the total duration for which the above cycle of sonication and interval should be done using the desired frequency. This is generally 30-45 minutes.
- Once this is done, sample container is collected and contents are put to centrifugation at 4°C.
- Collect the supernatant which contains the protein of interest.
- Proceed with further purification.

Schematic of sonicator is given below:

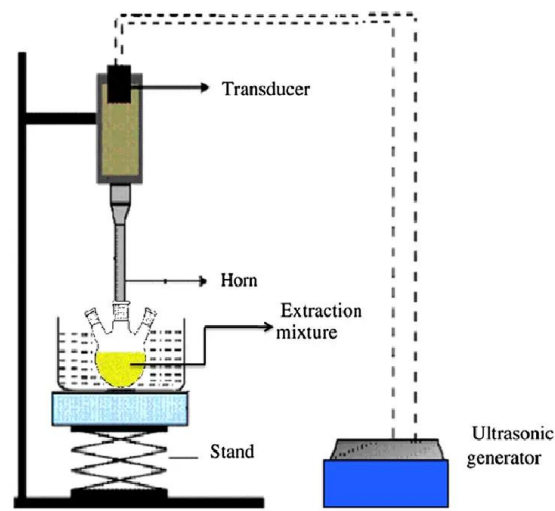
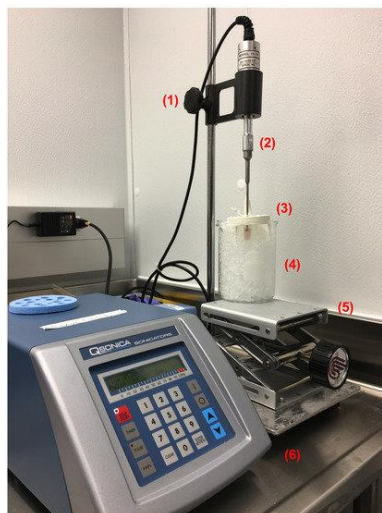


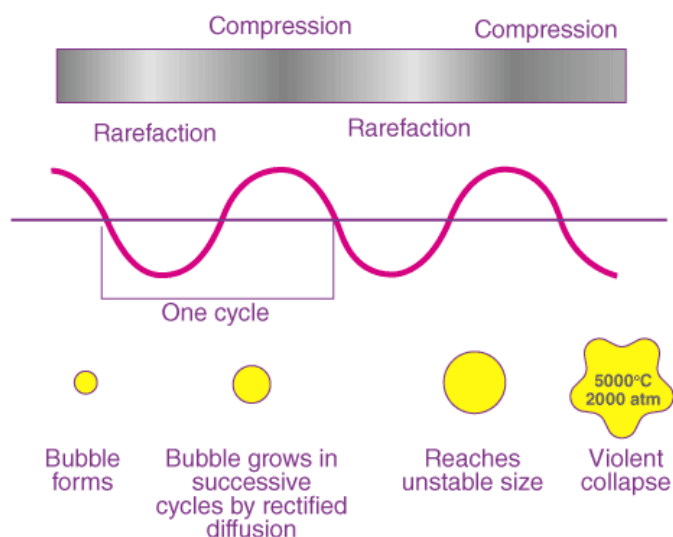
Image of actual sonicator is given below:



Sonication Equipment:

- (1) Rod and Clamp
- (2) Sonicator with Tip
- (3) Tube Holder
- (4) 600 mL Beaker with Ice
- (5) Adjustable Stage
- (6) Sonicator Control Box

Repeated cycles of sonication lead to formation of cavitation bubbles inside the cells. This leads to cells bursting from inside. This is shown in the figure below:



Note: Currently, we use a combination of both the methods to get better and quick results. In this, cells are incubated with lysozyme after resuspension in the presence of PMSF and after about 30 minutes of incubation the sample is cooled by placing it in ice bath and subjected to sonication.