Research Article (Open access)

Estimation of Mass of Single Cell

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ABSTRACT- Cells are always considered as a basic unit of life in every aspect, almost whole environment consists of various types of cells. Many scientists did research on such type of organisms and they concluded almost every function of how micro-organisms actually work in a proper manner. Every organism has a particular morphology, which can be a factor to differentiate organism based on size, but in this research, it has been found that how to measure the weight of single cell whether the prokaryotic or eukaryotic cell, independent of other factors. Thus, by finding the mass and relating to its size we can even find the density of organism, may be sometimes the size cannot be a factor to detect how heavy the cell is?. Hence, using this technique one can differentiate various organisms based on their single cell weight. Many applications can be found by the respective person if someone using this technique. One of the application is, one can isolate aerobic bacteria also using this technique and can sub-culture it.

Key-words- Cell size, Differentiate organisms, Isolation of aerobes, Single cell weight

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INTRODUCTION

The cell is the basic structural, functional and biological unit of all known living organisms. A cell is the smallest unit of life that can replicate independently and cells are often called 'the building blocks of life' [1]. Cells consist of cytoplasm enclosed within a membrane, which contains many biomolecules such as proteins and nucleic acids [2]. Organisms can be classified as unicellular or multicellular. While the number of cells in plants and animals vary from species to species. Cells are only visible under a microscope, with dimensions between 1–100 micrometers [3].

There are two types of cells: Prokaryotic cell and Eukaryotic cell. A prokaryote is a unicellular organism that lacks a membrane-bound nucleus, mitochondria or any other membrane bound organelle [4]. In prokaryotes, all the intracellular water soluble components (proteins, DNA and metabolites) are located together in the cytoplasm enclosed by the cell membrane, rather than in separate cellular compartments. A eukaryote is an organism whose cell contains a nucleus and other organelles enclosed within membranes [5].

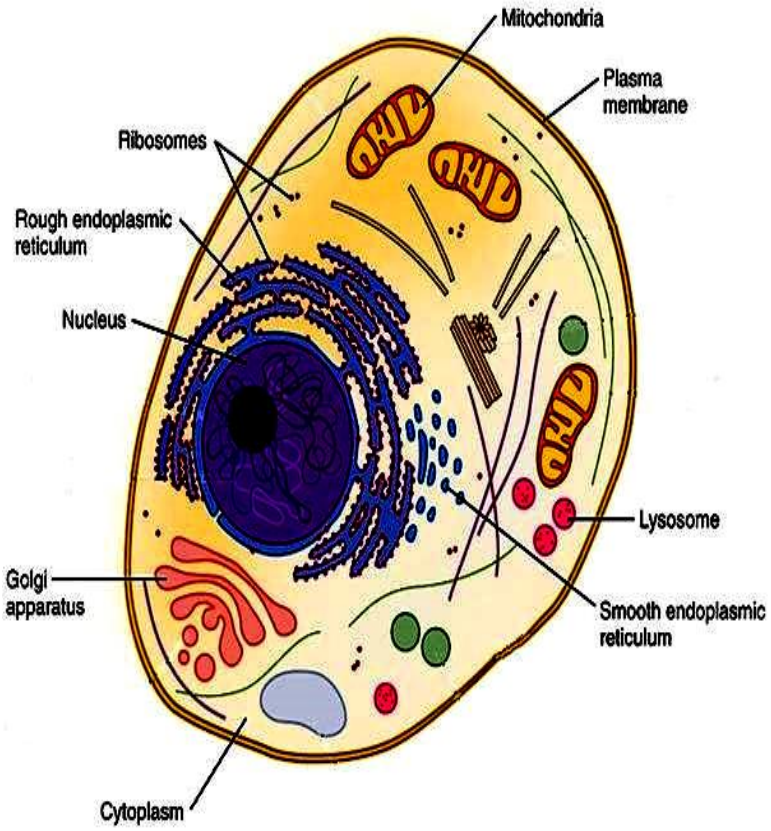

Fig. 1: Structure of Eukaryotic cell [6]

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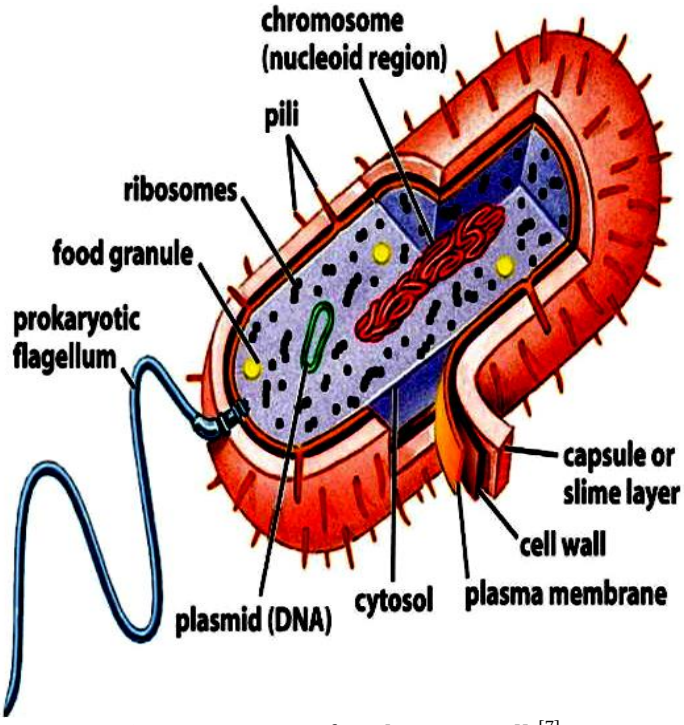

Fig. 2: Structure of Prokaryotic cell [7]

Silica gel is a granular, vitreous, porous form of silicon dioxide made synthetically from sodium silicate. Silica gel high specific surface area (around 800 m^2/g) allows it to adsorb water readily, making it useful as a desiccant. Silica gel is often described as "absorbing" moisture, which may be appropriate when the gel's microscopic structure is ignored, as in silica gel packs or other products. However, material silica gel removes moisture by adsorption onto the surface of its numerous pores rather than by absorption into the bulk of the gel [1].

Fig. 3: Silica Gel beads [8]

ESTIMATION OF MASS OF SINGLE CELL

AIM: To estimate the mass of single cell (Culture of interest) by Siddhesh's Method.

PRINCIPLE

The silica gel bead contains pores on it which can be used to grow the microbes in it. This technique consists of dipping the beads in the Nutrient broth and allowing the beads to grow the organisms in their pores, which were then weighed and by applying calculations mass of single cell can be estimated.

In this technique, silica gel beads firstly dipped in Nutrient broth so that some essential proteins required for microbial growth get entrapped in the pores of the beads. Then excess broth from the surface of the bead was removed with the help of ear cleaning cotton bud and a drop of specific microbial suspension, whose weight was to be determined the pour on bead with the help of micropipette (**Note**: Pore size on the bead > The size of organism, if we was taking *Staphylococcus aureus*, whose diameter was 1 µm then the pore size must be µm+n, where n= small increment in the diameter of pore size than organism's max length or diameter so that it can enter in pore, choose beads accordingly they are available in various types and mesh size) and kept in incubator at 37^0C for 1 hr. After one hour, microbial growth can be seen in silica gel beads with naked eyes and then by weighing the silica gel beads and applying calculations the mass of single cell can be estimated, which demonstrated in calculations portion.

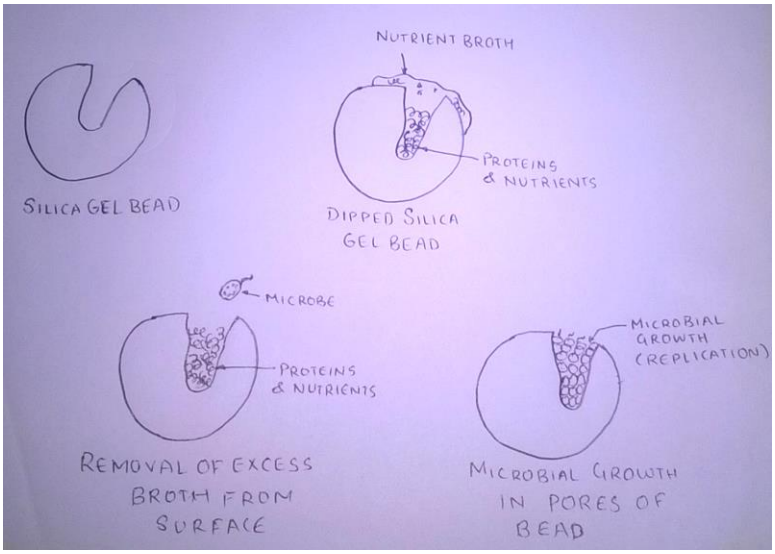

Fig. 4: Principle of Mass of Single cell

REQUIREMENTS

1. Sieved silica gel beads (2mm or as per requirement)
2. Microbial culture of interest
3. Ear cleaning cotton buds
4. Sterile plastic spoon
5. Nutrient broth (as per nutritional requirements of organism)

6. Petri plate dish
7. Micropipette
8. Distilled water
9. Sterile eppendorf tubes

PROCEDURE

Take sieved silica gel beads and keep in microwave for 5 mins at 120^{o}C so that the moisture present in the pore gets evaporated. Remove beads and weight to adjust ~1 gram as W_1. Dip these beads in the nutrient broth (liquid medium) in petri plate dish between burners (Beads should be totally sunk in broth). After 15 mins, remove beads with the help of a plastic spoon and take in a clean petri plate dish and remove the excess nutrient broth on the surface of the beads with the help of ear cleaning cotton bud. After cleaning, keep the beads for 5 mins between burners with a lid of dish open so that water molecules present on the surface gets evaporated. Take 5 µL culture suspensions and put on each bead and keep beads for 2 mins and removed the excess suspension around beads with the help of ear cleaning bud. Close the lid of petri plate dish and kept in the incubator for 1 hr at 37˚C. After 1 hr, microbes grow inside pores and beads turns white from transparent (Fig. 5). Weigh the beads W_2. Take one bead and put in distilled water in the eppendorf tube, when bead put on water bead has the greatest affinity towards water molecules and hence microbes present in pores gets displaced by water molecules and do cell counting under the microscope (volume of distilled water take as per required for cell counting technique). Now apply calculation as per shown in sample calculation portion.

OBSERVATION

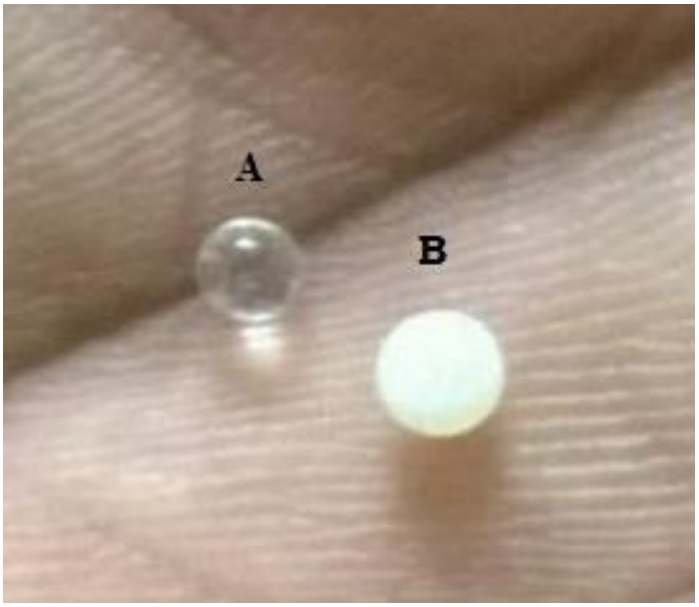

Fig. 5: Silica gel beads
A: Normal silica gel bead (Transparent)
B: Microbial growth in bead (white)

Fig. 6: Six Microbial beads kept in sunlight denoted by 1, 2, 3, 4, 5, and 6

Observation at shadows of beads:

Bead 1: Total microbial growth throughout bead hence no light passed through it;

Bead 2-4: Light passed through the nutrient broth (orange reflection in shadow) present in the centre of beads which resembles growth of microbes on the surface only

Bead 5: Shows partial growth of microbes due to depletion of nutrient broth and hence white reflection can be seen in shadow

Bead 6: No microbial growth in centre

(NOTE: For this experiment, all beads taken must be like Bead no. 1 after microbial growth hence, necessary to check under light)

CALCULATIONS

W_1- Mass of dry beads- 1.000 gram–Let 10 beads weight for 1 gram

W_2 - Mass of beads after microbial growth- 1.010 gram

Total microbial growth (TMG) in 10 beads= W_2–W_1

= 1.010-1.000= 0.01 gram

TMG in 1 bead= (TMG in 10 beads/No. of beads)= (0.01/10)=0.001 gram

After doing cell counting, there were 5 x 10^6 cells present in 1 bead

Then,

Mass of single cell= (TMG in 1 bead/No. of cells)= (0.001/5 x 10^6)= 2 x 10^{-10} gram

= 0.2 nanogram (ng) weight of single cell

CONCLUSIONS

The weight of single cell present in bead was 0.2 ng and hence using this technique weight of any organism can be found. If somebody wants to measure the density of particular single cell for example *S. aureus*, whose diameter was 1 µm, then the density of organism is given by (mass of single cell/ Volume of the sphere)= [m/(4/3) x πxr^3]. Hence, by using this technique we can differentiate various organisms based on their single cell weight.

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