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INSTRUMENTAL BASE OF MODERN TECHNOLOGIES FOR STUDYING DNA GENOMES

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Destruction of biological objects represents the first stage of an experiment and may include:
a) physical;
b) chemical (biochemical) destruction of cells or whole organisms.
Physical methods are most often used to destroy cells. In this case, the destruction of an object used to be carried out with the use of a mortar and pestle (kneading), then there appeared devices that crush these objects [18,19,20].

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Most animal cells are disrupted relatively easily, but significant difficulties are often encountered in disrupting plant and bacterial cells due to the presence of cell walls. Physical methods of cell disruption are subdivided according to whether it occurs by frictional forces between cells and solids (rubbing cells against solids) or hydrodynamically (cell disruption in liquid media). Modern science has a rich arsenal of crushers like mills, vibrating and ultrasonic tissue destroyers. Rubbing of cells with solid materials [1,2,3].

The modern modification of this method consists of grinding cells with sand or abrasive powder in a mortar with a pestle [5].

Nowadays, due to the advent of gentler methods of disruption, this method is rarely used for the disruption of animal cells, but it is still used for the disruption of plant and bacterial cells. It is desirable that the abrasive particles should be as sharp as possible and of the same size as the cells to be rubbed. The disadvantage of the method is that the structure of the largest organelles such as, for example, chloroplasts, may be disturbed when cells are rubbed. Squeezing cells mixed with abrasive particles through a Hughes press gives good results. Wet cells with abrasive particles are placed in a tube at -5°C, and then a single blow on the piston, creating a jump-like pressure change, pushes the cell mass through a narrow hole with a diameter of about 0.25 mm. A modification of this method is the pushing of cells at -25°C; the role of abrasive particles in this case is performed by ice crystals. To achieve maximum destruction of bacterial cells, it is sometimes necessary to increase the pressure up to 5.5×107 Pa [4,5,6,7,8,9].

Bacterial cells can also be destroyed by mechanical shaking of particle suspensions with abrasive powder at a frequency of 300-3000 vibrations per minute using a Mikl shaker in which small glass beads with a diameter of 50 to 500 µm are added. However, the strong vibration produced by shaking often causes destruction of cell organelles [10,11,12].

Destruction of cells in liquid media. Destruction of cells in suspension occurs either by rotation of the blades or piston (blenders) or by up and down progressive movement of the piston or balls (homogenisers). Blenders typically have cutting blades that rotate at high speed. The number and design of these blades varies, but they are all usually pointed at right angles to each other, and their shape ensures good mixing of the contents of the vessel [13,14,15].

The cell suspension is placed in a special beaker, which has sockets along the entire height and looks like a clover leaf in cross-section. To maintain a low temperature during homogenisation, the beaker is placed in ice. Due to the special arrangement of the blades and the design of the beaker, hydrodynamic forces are generated during fractionation. The method is quite versatile and is widely used for cell fractionation,

however, some undesirable effects may occur when the blades of homogenisers rotate rapidly [16,17,18].

Most homogenisers are an apparatus consisting of a pestle, either manually (Downs and Tönbreck homogenisers) or mechanically (Potter-Elwegeheim homogeniser) driven, which rotates or moves up and down in a cylindrical glass vessel. The gap between the pestle and the vessel walls must remain constant, as the rate of cell destruction depends not only on the speed of rotation of the pestle, but also on the ratio between the radii of the pestle and the vessel [19,20,21,22].

The vessel is fixed stationary, so the rotation speed of the suspension varies from minimum (at the vessel walls) to maximum (at the pestle surface). Consequently, the smaller the distance between these surfaces, the higher the velocity gradient. The forces arising at high velocities are sufficient to destroy rather thin membranes of animal cells, but plant and bacterial cells are not destroyed by homogenisers [23,24,25].

The efficiency of homogenisation depends to a large extent on the presence of vascular and connective tissue in the ground material, for the removal of which the sample is passed through a special meat grinder with holes of 0.88 mm diameter before homogenisation. 29 Polymorphonuclear leucocytes are destroyed by gentler methods - using a pipette. Eosinophils are destroyed by rapid passage under pressure through a fine-lattice sieve. Destruction of cells by high pressure. This method is mainly used for the destruction of microbial cells. Special presses are used for this purpose, such as the French Press, which pressurises the cells up to 10.4×107 Pa [2,4,5,6].

The cell suspension is loaded into the stainless steel chamber with the needle valve closed, through which the chamber is connected to the outside environment. Then the chamber is turned upside down, the valve is opened and the air is forced out of the chamber by the piston, after which the valve is closed again and the chamber is returned to its original position and placed on a fixed base [16,17,18,19].

By means of a hydraulic press the required pressure is applied to the piston; when a certain pressure is reached in the chamber, the needle valve is opened slightly, the pressure in the chamber is slightly reduced, and at this point the cells burst. The cell

mass flowing out of the chamber outlet is collected with the needle valve in the open position, maintaining a constant pressure in the chamber [20,21].
Unfortunately, it is not known what forces are generated in the chamber and how the cells and cellular components resist them [25].

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