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Lab scale centrifugation

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1 Abstract

In this document, centrifugation is introduced as a separation technique commonly used in laboratory practice, with preparative or analytical purposes. Then, centrifugation equipment and required components commonly used at lab scale will be presented. Finally, centrifugation techniques and their main applications will be distinguished.

2 Introduction

Centrifugation is a separation technique that consists of a sedimentation accelerated by means of a centrifugal force. It is a common unit operation used at industrial level in several industries such as the food industry, the pharmaceutical one and the biotechnological one. In the biotechnological context, it is applied to cells and mycelium separation (primary recovery), to separate the liquid containing a specific compound from the debris generated by cell disruption, or either to the separation of particle, cells, organelles or molecule mixtures.

Centrifugation is also a common technique used in the laboratory, it being applied both with preparative and analytical purposes. Regarding the former, the aim is to separate particles which will be further used for some purpose, whereas the latter is applied to determining some property of the particles being studied, such as the sedimentation rate or the molecular weight.

3 Objectives

After reading this document, the student will be able to:

- **Select the centrifugation technique** considered more appropriate for each application.
- **Discriminate among equipment, components and conditions** which are more adequate to undergo a particular separation.
- **Design** a centrifugal separation process which includes the stages needed for completing the desired separation.

4 Development

First, theoretical basis of centrifugal separation will be revised, to continue with a detailed description of centrifugal equipment and their characteristics. Then, centrifugation techniques used for laboratory purposes will be presented, particularly describing the aspects which distinguish differential centrifugation from density gradient centrifugation. All the previous giving examples of application in the biotechnological context.

4.1 Basis of centrifugal sedimentation

The study of **solid-liquid separation by centrifugation** is based in the sedimentation theory, which is explained by the **Stokes law** (Equation 1), which establishes the movement of a solid particle in a liquid medium when there is a density gradient between particle and liquid (Graham, 2001). It describes the sedimentation rate of a spherical particle in a continuous medium for Reynolds smaller than 1 (Tejeda et al., 2011).

The Stokes law establishes that, under specific centrifugation conditions, each particle sediments at a different rate as a function of its equivalent size and density. Larger and more dense particles will sediment first, the size being the parameter of larger influence on the sedimentation rate. From the Stokes law it is also deduced that the sedimentation rate increases by increasing the centrifugal force. This law may be applied to biological broths, they being considered diluted suspensions.

$$v_\omega = \frac{d_p^2 \Delta\rho \omega^2 r}{18\mu} \qquad \text{(Equation 1)}$$

Where:

v_ω: centrifugal sedimentation rate.
$\omega^2 r$: centrifugal acceleration.
ω: rotational speed in radians.
r: radial distance between rotational axis and particle.
d_p: particle diameter.
$\Delta\rho$: density gradient between particle and medium.
μ: medium viscosity.

4.2 Centrifugal equipment and components

At lab scale, the materials and equipment used are different to that employed at industrial level, although they work under the same physical principles. Lab scale centrifuges allow the control of rotational speed, time and temperature, and the available accessories also make it possible to modify tubes capacity as well as tube's shape and material. In general, conical and rounded centrifugal tubes are distinguished. In addition, Eppendorf tubes are frequently used in microcentrifuges, while Falcon tubes are common in bigger centrifuges.

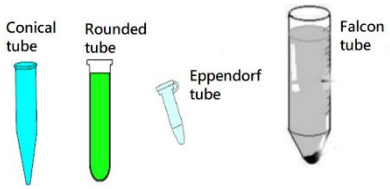


Figure 1. Tubes for centrifugal separations at lab scale (Técnicas de laboratorio en bioquímica. Centrifugación. http://biomodel.uah.es/).

There are fixed-angle and swinging head (or swinging bucket) centrifuges (fixed-angle rotor and swinging-head rotor, respectively). The former are designed to hold the sample containers at a constant angle relative to the central axis, while the latter have a hinge where the sample containers are attached to the central rotor, allowing the samples to swing outwards as the centrifuge is spinning. In some applications both

centrifuges can be used, whereas for specific applications one or other type might be more appropriate.

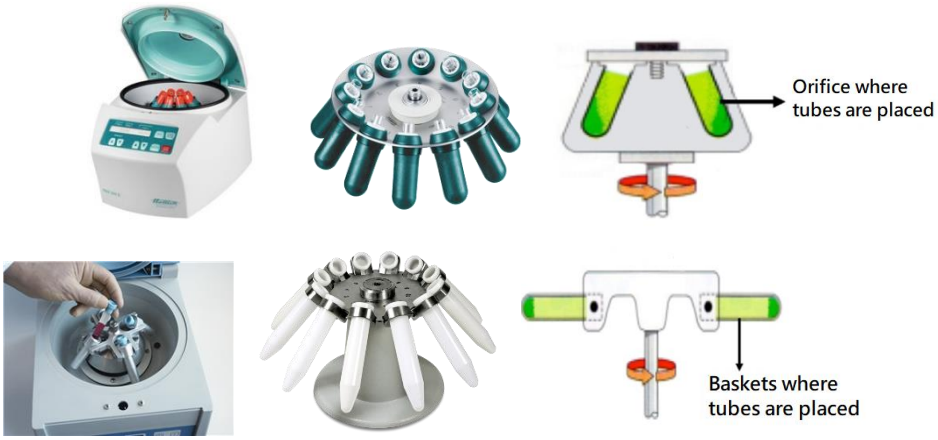


Figure 2. Upper raw: fixed-angle centrifuge, components and tubes position during spinning. Bottom row: swinging head centrifuge, components and tubes position during spinning.

4.3 Ways of conducting centrifugation

Centrifugation may be conducted in different ways. According to rotational speed, low speed centrifugation (20,000 rpm), high speed centrifugation (10,000-20,000 rpm) and ultracentrifugation (>20,000 rpm) are distinguished. In addition, centrifugation may have analytical purposes, with the aim of determining the physical properties of sedimenting particles; or either preparative purposes, with the aim of isolating the particles, cells or molecules of interest. Finally, there are different forms of conducting centrifugation with regard to the medium in which the separation takes place, and the way at which the sample is introduced in the centrifuge tube: differential centrifugation and density gradient centrifugation.

4.4. Differential centrifugation

Differential centrifugation takes place when a sample is introduced in the centrifuge tube and it is centrifuged at a specific speed. Behavior of particles in the medium will depend on their size, shape and density, as established by the Stokes law (Equation 1), as well as on the centrifugation conditions. At a specific rotating speed, each particle will sediment at a specific rate, which is a function of its density and equivalent size. Bigger and denser particles will sediment first, followed by less dense and smaller particles (Figure 4). Therefore, in differential centrifugation, not only rotating speed but also centrifugation time will determine particle separation. When centrifugation is finished, a pellet and a supernatant are obtained.

Differential centrifugation may be used to separate cells, given that a cell suspension subjected to successive cycles of increasing centrifugal force will yield a series of pellets containing cells of decreasing sedimentation rate.

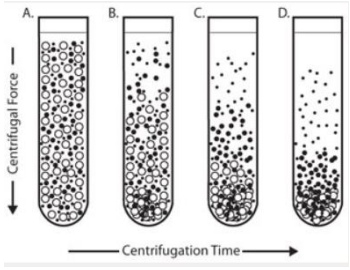


Figure 3. Differential centrifugation (Mark Frei, BioFiles).

A classical application of differential centrifugation is cell fractionation, which consists of separating the main (sub)cellular organelles from a homogenized cell suspension, i.e. a suspension previously subjected to cell disruption. It is not common to apply more than four cycles of centrifugation to separate subcellular organelles

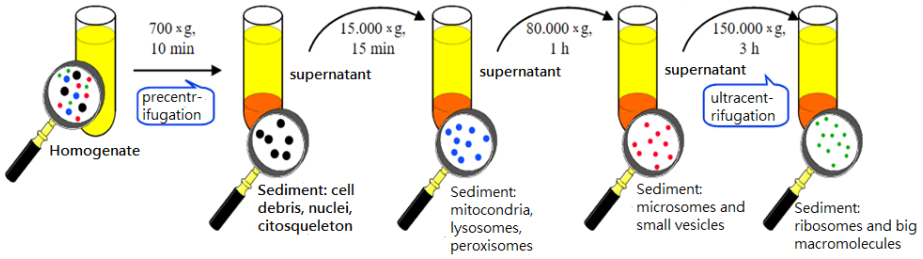


Figure 4. Cell fractionation by differential centrifugation (http://biomodel.uah.es/).

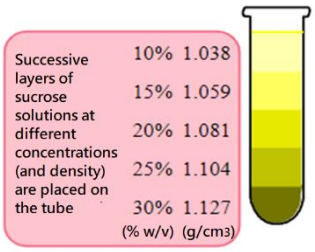
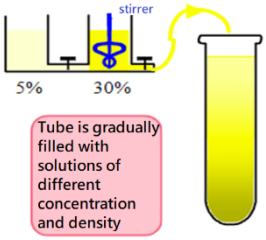
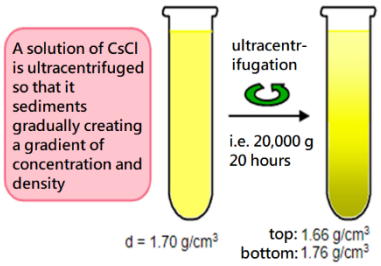
Nevertheless, due to biological particles heterogeneity, differential centrifugation might not be successful, or the sediments might be contaminated with particles different from the desired ones. In this case, the sediment can be washed by a re-suspension and re-centrifugation procedure. To perform cell fractionation, however, there is usually a need for density gradient centrifugations.

4.5. Density gradient centrifugation

A **density gradient centrifugation** implies the use of a medium which varies in density along the centrifuge tube. This centrifugation technique is performed in swinging-head rotor centrifuges. Density gradient centrifugation can be classified into **rate-zonal** and **isopycnic centrifugation**.

The density gradient is created thanks to a concentration gradient that consists of preparing growing concentrations of an appropriate component along the centrifuge tube. To this end, different components such as sucrose, cesium chloride, bovine serum albumin, or some commercial media such as Ficoll® and Percoll® are used. A discontinuous gradient can be created manually, a special device can be used to create a continuous one, or the gradient can be self-generated by centrifugation, usually while fractionating the sample (Table 1) (Técnicas de laboratorio en bioquímica. Centrifugación. http://biomodel.uah.es/).

Table 1. Density gradient types. Examples of preparation (Adapted from: Técnicas de laboratorio en bioquímica. Centrifugación. http://biomodel.uah.es/)

PREFORMED GRADIENT		SELF-GENERATED GRADIENT
Discontinuous gradient	**Continuous gradient**	**Continuous gradient**
Example of preparation, by mixing different solutions (valid for zonal and isopycnic, depending on chosen densities and centrifugation time). 	Example of preparation, using a mixture of two solutions in a gradient formation device. 	Example of preparation by centrifugation. The gradient is formed while sample components are being separated (The example, CsCl, is not used for cells but for nucleic acids). 

4.5.1. Rate-zonal centrifugation

Rate-zonal centrifugation consists of layering the sample as a narrow layer on top of a density gradient. Consequently, as centrifugation proceeds, there is a classification based in sedimentation rate differences so that the faster sedimenting particles are not contaminated by the slower ones, as occurs in differential centrifugation. Particles concentrate in discrete regions or bands. The gradient stabilizes the bands generated during centrifugation and provides a medium of increasing density and viscosity (Figure 5).

Sedimentation rate is the separating mechanism, which depends mainly on their size and shape. This allows better separation of particles having different sizes, thanks to the density gradient formed. The created bands will be carefully collected by sampling the tube, or by making a hole in the base of the tube. Since the density of the particles is greater than the density of the gradient, all the particles will eventually form a pellet if centrifuged long enough. Therefore, rate-zonal centrifugation must come to an end before the larger components reach the tube bottom. In figure 6, a detail of the stages undergone in a rate-zonal centrifugation procedure are shown.

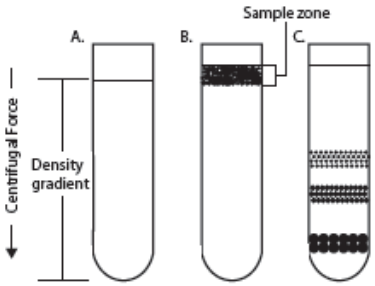


Figure 5. Rate-zonal centrifugation (Biofiles online. Sigma.com/biofiles)

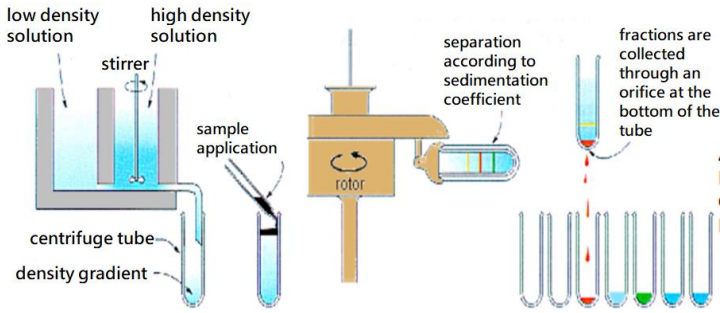


Figure 6. Stages of rate-zonal centrifugation. (A) Creation of a density gradient with an automatic device (B) Placing the sample on the gradient at the top of the tube (C) placing the tubes in the swinging-bucket rotor and centrifugation (D) collecting the pellets separated in bands (Source: Técnicas de laboratorio en bioquímica. Centrifugación. http://biomodel.uah.es/).

4.5.2. Isopycnic or equilibrium centrifugation

In **isopycnic centrifugation**, also called **buoyant or equilibrium separation**, particles are separated solely on the basis of their density. It also uses a density gradient, but the main difference with rate-zonal centrifugation is that the sample is centrifuged long enough to reach sedimenting equilibrium. Particle size only affects the rate at which particles move until their density equals that of the surrounding gradient medium; therefore, the separating principle will not be the sedimentation rate but density.

Isopycnic centrifugation implies the gradient being used to have a higher density than the denser particles in the suspension. Continuous gradients covering the whole range of densities are required. Thus, independently on the sedimentation time, particles will never reach the tube bottom, but they will reach a stable equilibrium in the middle of the gradient, where a very thin layer of higher resolution is formed (Figure 7). In isopycnic centrifugation the gradient is usually a self-generated one: the sample is mixed with the gradient and ultracentrifugation speed is applied to undergo separation.

Apart from the high resolution obtained by this method, the interesting point of this technique consists of the fact that, upon centrifugation, particles of a specific density sediment until they reach the point where their density is the same as the gradient media (i.e., the equilibrium position). The gradient is then said to be isopycnic and the particles are separated according to their buoyancy (isopycnic = similar density, from the Greek). It is mainly applied to nucleic acids separation and purification.

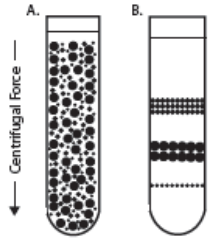


Figure 7. Isopycnic centrifugation (Biofiles online. Sigma.com/biofiles)

5 Closing

In this learning object we have revised the centrifugation theory and how it can be applied at lab scale in the biotechnological context. In particular, centrifugation equipment and most common centrifugation techniques used in lab practice have been presented.

Applying what you have learned, ¿Would you be able to identify which centrifugation techniques are represented in the drawing below? (Figure 8)

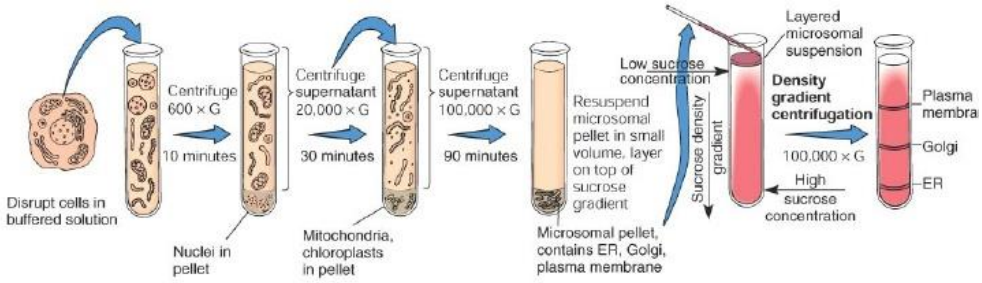


Figure 8. Cell fractionation by centrifugation. Adapted from de Biology, Seventh Edition CHAPTER 4 Organization of the Cell fractionation Copyright © 2005 Brooks/Cole - Thomson Learning. Available onlineon https://slidetodoc.com/biology-seventh-edition-solomon-berg-martin-chapter-4/

To finish, you are invited to make a comparative table of the different centrifugation techniques studied including:

a) A drawing of the tube before and after separation.

b) Rotor type.

c) Specify the use (or not) of a density gradient. Gradient type, if used.

d) Main separation principle (speed, density, size, time).

e) Main application of the technique

6 References

Centrifugation Separations. Merck. Mark Frei, BioFiles v6 n5.

Centrifugation. Biofiles online. Sigma.com/biofiles.

Graham, D.J. (2001). Biological Centrifugation (1st ed.). Garland Science. https://doi.org/10.1201/9781003076797

Methods of Cell Separation. Laboratory Techniques in Biochemistry & Molecular Biology. Elsevier Science, 2012.

Solomon, Berg, Martin. (2005) Biology, Seventh Edition Ch4. Organization of the Cell. Fractionation. Brooks/Cole Thomson Learning

Técnicas de laboratorio en bioquímica. Centrifugación. http://biomodel.uah.es

Tejeda, A., Montesinos, R.M. y Guzmán R. (2011). Bioseparaciones (2ª Ed). Capítulo II.4. Pearson Educación de México.