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Review

Foaming of proteins: New prospects for enzyme purification processes

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ABSTRACT

Efficient techniques for the isolation of enzymes from a microbial production culture are required to meet the growing needs of the "White Biotechnologies" for novel catalysts. Traditional protein purification procedures typically comprise multistep operations, which inevitably come along with significant losses of enzyme activity. Foaming offers an alternative minimizing the processing steps, preserving the purification efficiency and decreasing the activity losses all at the same time. This review provides an insight into the foaming process itself and its application in separating enzymes from model systems and from complex media, such as microbial cultures. Examples demonstrate fractionated foaming and the tweezer technique.

Contents

1. Introduction

The catalytic properties of enzymes were applied long time before their nature and capabilities were recognized, for example for preparing bread, cheese, alcoholic beverages, and in the manufacture of leather. The historic beginnings of enzyme technology go back to discoveries in the late 19th century. Buchner (1897) verified the existence of a so-called "ferment" which showed a catalytic activity not bound to vital cells. Until now, about 4200 enzymes are listed in the *Enzyme Nomenclature Database*, but only an estimated 50–100 are used on a larger industrial scale. In principle, every organism is a potential source of enzymes, but for industrial applications both enzyme stability and yields must be adequate. After a new enzyme activity is located, it has to be characterized on the biochemical and molecular level. To this end, the enzyme has to be purified from all associated cell constituents, such as other proteins (especially peptidases), salts and inhibitors produced during the culture period.

The main problem covering all over the down-streaming process is the irreversible loss of the 3D-structure of an enzyme, resulting in a loss of catalytic activity. Increasing the number of separation operations for improved refining means to accept the concomitant

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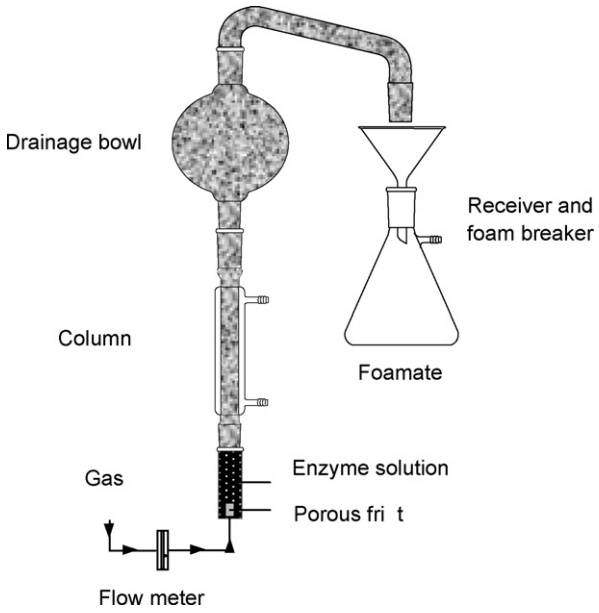

Fig. 1. Scheme of a foaming device.

reduction of activity with each step. Considering these problems there is an obvious demand for efficient alternatives for enzyme purification with sufficient selectivity, maximal recovery, adequate enrichment, and high preservation of enzyme activity besides acceptable costs and minimal time expense. Preparative foaming, also called adsorptive bubble separation (ABS) or foam fractionation represents a promising option. The principle was described and patented already in 1920 (Ostwald, 1920), but has experienced a renaissance in recent years. Foaming is a method to separate soluble and surface-active compounds from diluted aqueous solutions (Uraizee and Narsimhan, 1990). Bubbling gas into a liquid containing the target molecule results in a formation of a gas–liquid interface, at which bipolar compounds arrange in a definite formation and are then transported upwards with the emerging foam (Loha et al., 1997). By using an inert gas the foaming procedure can be adjusted to the requirements of biomolecules sensitive towards oxidation. The method is distinguished by mild conditions of operation, minor energy and investment costs, lack of organic solvents, little time consumption and the simple construction of the devices (Fig. 1) (Montero et al., 1993). Altogether, the environmentally compatible mode of operations predestines preparative foaming as a partner for the integration in white bioprocesses.

2. Principles of foaming

Lemlich (1968) classified foaming into adsorptive bubble separation (ABS) methods comparable to flotation. Differences between both methods relate to the separation of coarse-particle molecules with a floating foam (flotation), whereas in foaming dissolved surface-active substances adsorb at an interface area.

First investigations of foaming of proteins were confined to the quantitative transport of constituents out of press juice of potatoes or sugar beet (Ostwald and Siehr, 1937). It was quickly realized that foaming holds promise, and therefore it was adapted to the selective transport of biomolecules out of multi-component systems. After selection of optimal conditions, gluten was separated selectively from starch (Rodgers, 1972). Extending the range to natural products, dyes were produced from Kava Kava (Backleh et al., 2003a), and antioxidants were isolated from rosemary (Backleh et al., 2003b). Environmental applications were reported for the treatment of wastewater and removal of heavy metal ions (Nii and Kinoshita, 2009).

The method is based on the adsorption of surface-active compounds to the interface of a gas–liquid dispersion after passing gas through a solution containing the target molecules. By self-orientation and formation of cohesive films the bipolar molecules stabilize the dispersed gas phase. While the hydrophobic parts of the molecules attach to the gas phase, the hydrophilic parts are oriented towards the liquid phase. The foam rises up in a column, while physical processes, such as drainage and coalescence decrease the foam volume continuously because of the backflow of excessive liquid. Thereby the adsorbed target molecules are enriched in the foam phase. Non-adsorbed solutes, which are transported in the liquid phase, drain down along the lamella of the foam bubbles into the retentate. Fractions of molecules differing in hydrophobicity develop along the foam column; thus, repetitive formation of equilibria is obtained as in chromatography (Maas, 1973). The foam is collected at the end of the column and re-liquefied, for example by the application of a weak vacuum, by ultrasound, or by stirring. To optimize the foaming process for the separation of enzymes, it is essential to know the configuration of the different foam phases, the physical effects influencing the foam column, and the physical–chemical properties of the target proteins/enzymes.

2.1. Formation of foam

Foams are dispersions of gas in a liquid. Their formation is a physical process, which is divided into three phases:

(I) The initial phase is the generation of gas bubbles into the lower part of a liquid phase by bubbling gas through a porous frit. The homogeneity of the bubble sizes is ensured by a defined pore size of the frit. The smaller the pore size, the more homogeneous is the bubble swarm (Narsimhan and Ruckenstein, 1986).

(II) The gas bubbles rise up through the liquid phase due to the density difference, while surface-active compounds such as proteins/enzymes adsorb to the gas–liquid interface. This decreases the surface tension of the liquid, and the formation of foam is initiated (Damodaran, 1997). The adsorbed molecules stabilize the gas bubbles due to the formation of a membrane-like, visco-elastic film around the bubbles. Intermolecular and non-covalent interactions act within the same interface as well as between adjoining bubbles.

(III) Finally, the gas bubbles leave the liquid phase and cover its surface. The foam column grows.

2.2. Adsorption of surface-active compounds to the interface

Surface-active compounds adsorb to the gas–liquid interface in a defined arrangement. The velocity of adsorption is under control of diffusion. In the steady state the concentration of adsorbed molecules at the interface is higher than that of non-adsorbed in the liquid phase. The adsorption occurs in consequence of energy differences. Biomolecules, such as enzymes, are encircled in aqueous phases by water clusters and constitute a regular structure. At the interface the adsorbed molecules lose some of the hydration water molecules and consequently, receive an increase of entropy, whereby the process of adsorption becomes exergonic (Damodaran, 1997). According to Gibbs's equation of adsorption the graphical design describes a Langmuir isotherm related to a monolayer of adsorbed molecules (Graham and Phillips, 1979). Amongst others, the adsorption to the interface is defined by the concentration of molecules. The optimal concentration for most effective adsorption ranges from 10^{-7} to 10^{-3} mol L^{-1} (Maas, 1973). Values above the critical micellar concentration (cmc) cause an adverse effect. The molecules form micelles spontaneously and cannot contribute to the foam generation (Charm et al., 1966). Foam