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# Yeast cell disruption strategies for recovery of intracellular bio-active compounds — A review

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ABSTRACT

Yeasts are cheap, attractive and easily available residual sources of valuable bio-active compounds. Extraction of these compounds requires to break the yeast cells. So efficient damage of cell wall has become an important issue to be resolved. The aim of this paper is to review the potential of some emerging cell disruption techniques for recovery of intracellular bio-active compounds from Baker's yeast including mechanical (bead mill, high pressure homogenization, ultrasonication), and non-mechanical (electrical, physical, chemical and enzymatic) techniques, as well as some newly developed methods. The advantages and drawbacks of different cell disruption methods were summarized by considering the energy consumption, the interaction of the disruption methods with downstream operations and the process economics of alternative strategies. Finally, some future directions for research areas are proposed.

*Industrial relevance:* Wine making process entails the generation of significant amount of waste yeast, which represents an attractive source of valuable compounds that has been relatively unexploited to date. To retain the valuable cell content, effective cell disruption strategies are needed to break the rigid yeast cell walls. This review summarizes the state of the art of some emerging cell disruption techniques for recovery of intracellular bio-active compounds from yeasts including mechanical (bead mill, high pressure homogenizer, ultrasonication), and non-mechanical (electrical, physical, chemical and enzymatic) techniques. Thereby, it identifies the process economics of alternative strategies by considering the interaction of the disruption methods with downstream operations as well as the current situations and future research needs.



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## 1. Introduction

The yeast represents an attractive source of valuable compounds that has been relatively unexploited to date. In fact, the interior of yeast cell is a rich source of bio-active compounds (proteins, cytoplasmic enzymes, polysaccharides, etc.) valuable in biotechnology, pharmacology and food industry (Walker, 1998). Like other microorganisms, yeasts are surrounded by rigid cell walls that have to be disrupted in order to obtain the valuable cell content (Klis, 1994). Some conventional disruption methods including electrical, physical, chemical and enzymatic treatments are employed and achieved selective release of these bio-active coumpounds (Harrison, 1991; Middelberg, 1995; Yusaf & Al-Juboori, 2014). Electrical methods are based on breakdown or disruption of the cell by applying electric fields and the releasing profiles of target conpounds depend on the electric conditions (Vorobiev & Lebovka, 2006). Physical methods rely on disruption of the wall structure without micronisation of cell debris for separated release of soluble proteins, enzymes or other cell content. Chemical methods rely on permeabilisation of the outer cell wall or membrane by a large variety of chemical compounds, which allows periplasmic product to seep through mannoprotein complex of the yeast cell wall (Middelberg, 1995). Enzymatic methods are usually conducted in the presence of an enzymatic lysis that disrupts the outer membrane by enzymatic attack of the yeast wall (Harrison, 1991). These non-mechanical methods are gentle and usually result in small intracellular product release, however, the use of chemicals and enzymes can be problematic and lead to a higher degree of complexity of the downstream process operations. Besides, these non-mechanical methods are often limited to a small scale, owing to their limited general applicability and in most cases, low process economics or efficiency. Consequently, they have found limited commercial application to date (Geciova, Bury, & Jelen, 2002; Günerken et al., 2015).

The limitations associated with the non-mechanical methods have inspired numerous investigations into other alternative methods for efficient recovery of intracellular products from yeasts. Mechanical methods including bead mill (Woodrow & Quirk, 1982) high-pressure homogenization (Clarke, Prescott, & Khan, 2010),and ultrasonication (Chemat, Huma, & Khan, 2011) are most widely used to achieve microbial cell disruption for intracellular product release at an industrial scale (Harrison, 1991; Middelberg, 1995). These methods result in considerable cell breakage and high recovery of bio-active compounds. However, most of the mechanical methods are non-selective, structural elements of the cell are disintegrated and essentially the entire contents of the cytoplasm and cellular organelles are released (Geciova et al., 2002). Besides, the target products are subjected to mechanical stresses that cause cell disruption, which may have a possibility to affect the biological activity of the target products (Chisti & Moo-Young, 1986). In order to overcome these limitations, the application of some combined methods or newly developed technique was proposed. Most of the combinations are in the form of mechanical disruption with non-mechanical pretreatment, with several studies available in the literature (Baldwin & Robinson, 1990; Harrison, Dennis, & Chase, 1991; Shynkaryk, Lebovka, & Lanoiselle, 2009). Improved product recovery with a reduction in the energy requirement was achieved by combined methods. Besides, several new developments or new technologies for cell disruption including laser treatment (McMillan, Watson, Ali, & Jaafar, 2013), ionizing radiation (Lado & Yousef, 2002) and nanoparticle treatment (Chen et al., 2009) are emerging rapidly, aiming at reaching a better cell disruption efficiency.

Identification of a suitable cell disruption method is very important to accommodate efficient and cost effective recovery of bio-active compounds from the impermeable cell wall and membrane of yeast or other macrobial cells (Asenjo, 1990; Balasundaram, Harrison, & Bracewell, 2009). Several reviews on cell disruption techniques are available, including one by Harrison (1991) and one by Middelberg (1995), which discuss process-scale disruption approach of microorganisms in detail. Some newly published reviews discussed the disruption method of microbial cells for agricultural application (Yusaf & Al-Juboori, 2014) and microalgae biorefineries (Günerken et al., 2015). This review will be mainly involved with yeast cells due to their rich source of bio-active compounds valuable in food and health industries. Therefore, the purpose of this review is to update and condense a representative advancements in yeast cell disruption strategies for effective release of intracellular bio-active compounds, and provide some viewpoints on the current situation and suggestions for future research directions.

## 2. Yeast cell structure

Yeast cells exhibit great diversity with respect to cell size, shape and colour due to different external physical and chemical growth conditions including cultivation temperature, presence of some chemical compounds, and composition of the growth medium or growth phase (Walker, 1998). Among different yeast species and culture conditions, the composition of yeast may vary widely. In the following we will concentrate on *Saccharomyces cerevisiae* (*S. cerevisiae*).Table 1 presents a typical composition found in Baker's yeasts, which comprise mainly proteins, glycoproteins, polysaccharides, polyphosphates, lipids, and nucleic acids (Feldmann, 2005). These intracellular compounds are retained at various locations within the cell including cell wall, periplasm, plasma membrane and cytoplasm (Balasundaram et al., 2009). The plasma membrane, periplasmic space, and cell wall form the cell envelope (Fig. 1), which takes about 15% of the total cell volume and serves as a protecting capsule in controlling the permeability of the cell. Therefore, knowledge of the yeast cell envelope structure is essential in selecting a proper disruption method and disruption condition to accommodate efficient and cost effective release of these bio-active compounds. The rigid cell wall of yeast is a remarkably thick (100 to 200 nm) envelope that represents 20–25% of the dry weight of the cell (Lipke & Ovalle, 1998). The major structural constituents of the yeast cell wall are polysaccharides (80–90%), mainly glucans and mannans, and a minor percentage of chitins and proteins (Kollar et al., 1997). Glucans form a microfibrillar network primarily composed of β-2.6 and β-1.3-linkages, providing strength to the cell wall. Mannans are formed by a backbone of mannose residues in α-(1–6) linkage with short oligosaccharide side chains (Engler, 1985). Chitin is a polymer of *N*-acetylglucosamine representing only 2–4% of the dry weight of cell wall and mainly located in bud scars. Proteins found in yeast cell walls constitute the innermost part of the cell wall and give the cell its shape. Other components of the cell wall are variable quantities of lipids and inorganic phosphate. The plasma membrane with thickness of about 7 nm separates the interior of the cell from the extracellular environment. It is a thin semi-permeable lipid bilayer formed mainly by proteins and lipids, which plays a vital role protecting the integrity of the interior of the cell by selective permeability, i.e. to control what enters and what leaves the cytosole (Feldmann, 2005). The periplasm is a thin region between plasma membrane and the cell wall. It mainly contains secreted proteins (mannoproteins) that are unable to permeate the cell wall and the plasma membrane (Walker, 1998).