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MINI-REVIEW

Pressure to kill or pressure to boost: a review on the various effects and applications of hydrostatic pressure in bacterial biotechnology

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Abstract Much knowledge has been gained for the last 30 years about the effects of pressure on bacteria, and various pressure-based technologies have been designed. The development of modern molecular biology techniques (e.g., DNA microarrays) as well as the technological advances realized in the manufacturing of robust sampling and high-pressure devices has allowed these advances. Not only the direct effects on cell components (membranes, proteins, and nucleic acids) have been unraveled, but also the cellular response to pressure has been investigated by means of transcriptome and proteome analyses. Initially, research was performed by marine biologists who studied the microorganisms living in the deep sea at pressures of 1,000 bar. In parallel, food technologists developed pressure-based methods for inactivating microorganisms without altering the food properties as much as with temperature treatment. The preservation of specific product properties is also the rationale for pressure-based methods for the disinfection of biomaterials and for vaccine production. Therefore, attention was first focused on the "killing" potential of high pressure. On the other hand, there has been a growing interest in using elevated pressures (up to ~10 bar) for enhancing the productivity of bioprocesses. In this case, no killing effect was sought, but pressure was applied to "boost" the process by enhancing the oxygen transfer to the cell culture. This paper gives an overview on the effects of pressures in the range of 1 bar to 10 kbar on bacteria and presents the major and most recent achievements realized in the development of pressure-based biotechnological applications.

Keywords Pressure · High-pressure bioreactor · Bioprocesses · Food processing · Biomaterial disinfection · Protein refolding

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Introduction

Even though one may be under the impression that pressure does not vary much from 1 atm (1.025 bar) in nature, high pressures in the order of several hundreds of bar can be easily encountered in the deep sea since the hydrostatic pressure increases by about 1 bar per 10 m. The deep sea starts at a depth of 1,000 m (100 bar) and represents 75% of the total volume of the oceans and ~62% of the global biosphere (Fang et al. 2010). Despite harsh conditions, a surprisingly rich microbial community of the so-called piezophiles inhabits this environment. The majority of these piezophiles are Gram-negative facultative anaerobic and psychrophilic bacteria that face not only high pressure but also lack of nutrients and cold temperature (2–3 °C), except for the ones living close to hydrothermal vents and supporting temperatures up to 400 °C (Fang et al. 2010).

Apart from this natural case, microorganisms can be exposed to high pressure in industrial processes, which are used in their majority for inactivating pathogens in food products. Scientists from marine biology and food technology thus led most of the research about high-pressure effects on microorganisms and focused mainly on pressures higher than 200 bar and on their bactericidal effect. Yet, there has been a growing interest in applying elevated pressure in the range of 1–10 bar during bioprocesses in order to increase the oxygen transfer rate (OTR) and, as a result, achieve higher cell densities. The potential of these elevated pressures to "boost" bioprocesses is very promising but still requires deeper knowledge about their effects on microorganisms. Indeed, studies on elevated pressure are still far scarcer than the ones on high pressure.

An overview on the effects and applications of both elevated and high pressure that are discussed in the following is given in Fig. 1.

General concepts

Effects of high pressure are driven by volume changes

Most of the effects of pressure on microorganisms can be explained by the relation between pressure and volume change. Therefore, a short summary of the thermodynamic fundamentals behind it is given here. Pressure, like temperature, is an important thermodynamic variable, and its effect on molecular systems can be predicted based on Eq. 1, where G is the Gibbs free energy, p is the pressure, T is the temperature, K is the equilibrium constant, R is the ideal gas constant, and V is the volume.

$$\left(\frac{\partial \Delta \mathrm{G}}{\partial \mathrm{p}}\right)_{\mathrm{T}} = \left(\frac{-\partial \ln \mathrm{K}}{\partial \mathrm{p}}\right)_{\mathrm{T}} \mathrm{RT} = \Delta \mathrm{V}. \quad (1)$$

This equation, similarly as the Le Châtelier–Braun principle, states that when pressure increases, a given equilibrium will be shifted to the side that occupies the smallest volume (e.g., molecules A and B in Fig. 2). In case of a non-equilibrium process, the pressure dependence of the reaction rate k is given by the activation volume $\Delta V^{\neq}$ (Eq. 2)

$$\left(\frac{-\partial \ln k}{\partial p}\right)_{T} RT = \Delta V^{\neq}. \quad (2)$$

Increasing the pressure can thus either accelerate or decelerate reactions depending on the sign of the activation volume $\Delta V^{\neq}$. This feature contrasts with the effect of temperature increase that is always accelerating (cf., Arrhenius law).

Concerning the effect of pressure on chemical bonds, the covalent bonds are not affected, at least not up to 10 kbar (Mozhaev et al. 1996). Therefore, the structure of small

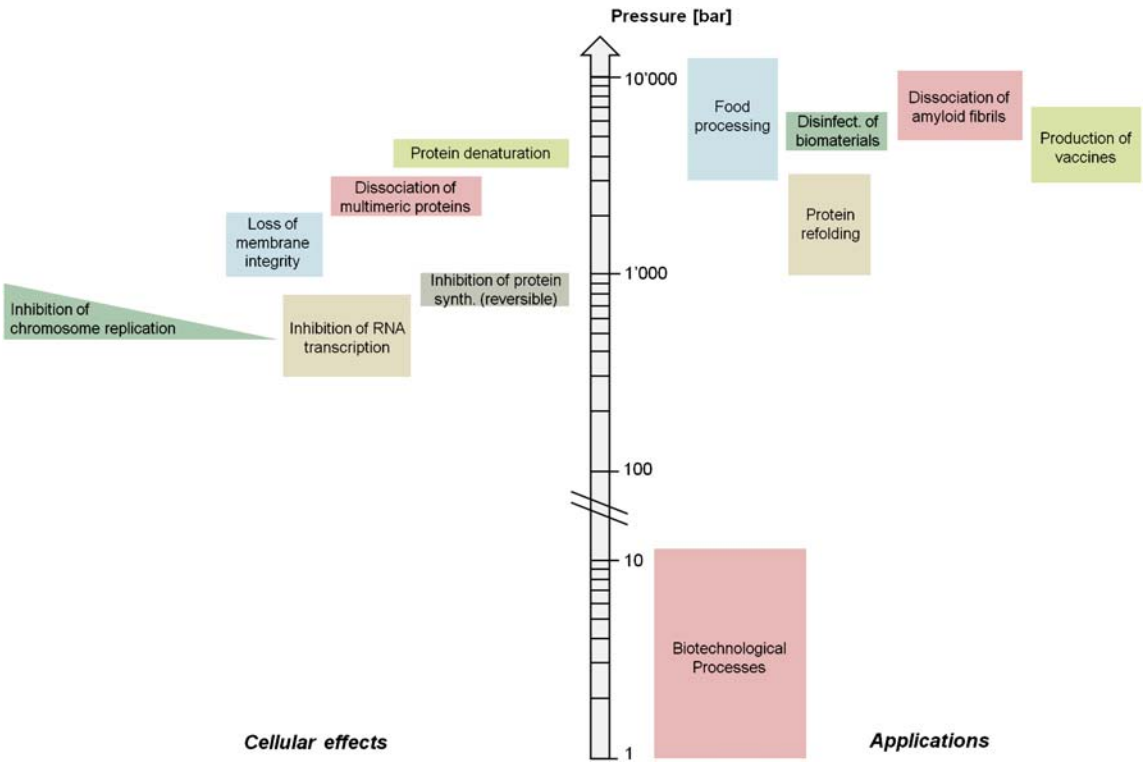

Fig. 1 Overview on the effects of pressures in the range of 1 bar to 10 kbar on microorganisms (*left*) and on their major applications in biotechnology (*right*). The range of pressure corresponding to each effect and application is expressed by the height of the form surrounding the text description

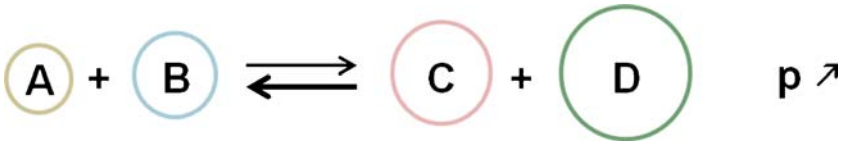

Fig. 2 Shift of equilibrium state upon pressure increase towards the species that occupy the smallest volume (*left*)

molecules (peptides, lipids, saccharides) and the primary structure of macromolecules (proteins, nucleic acids, polysaccharides) are not expected to change. In contrast, hydrogen bonds are stabilized by high pressure, whereas dissociation of neutral molecules in ions and exposure of charged groups towards aqueous medium are both favored, the overall volume being reduced through better hydration (= electrostriction) (Meersman and Heremans 2008). Since pressure affects essentially weak bonds, it can modify the conformation of macromolecules, as well as their interactions, and therefore possibly alter their function.

Effects of elevated pressure are mainly indirect effects caused by the increase of gas solubility

Pressures of elevated range (up to ~10 bar) are far too low to cause noticeable effects on molecular systems. However, they may indirectly affect microorganisms by inducing variations in the dissolved gas concentrations, in particular for O_2 and CO_2 (see the following discussion). Pressure influences the solubility of gases according to Henry's law, which states that "*at a constant temperature, the amount of a given gas that dissolves in a given type and volume of liquid* (c_g) *is directly proportional to the partial pressure of that gas in equilibrium with that liquid* (p_g)" (Eq. 3, with K_H being the Henry constant, specific to the gas dissolved).

$$p_g = K_H \cdot c_g. \tag{3}$$

It has to be noted that since this law was derived for ideal conditions, some deviations can be expected for large gas concentrations. In addition, gas solubility can change if the gas reacts with the solvent and in culture broths that contain various chemical compounds (Schumpe et al. 1982) and a large number of cells. The increase of gas solubility with pressure is especially relevant for processes that run at elevated pressure but not so much for those that run at high pressure (> 200 bar) since they are usually performed in degased systems.

Effects of high pressure on bacteria (> 200 bar)

Cellular components

Nucleic acids are the most stable cell components under high pressure due to the additional stabilization provided by hydrogen bonds. If this seems at first sight positive, it also suggests that the replication and transcription of DNA that require the formation of single-strand DNA may become more difficult upon pressure increase (Macgregor 2002; Oger and Jebbar 2010). Phospholipidic membranes, in contrast, are quite sensitive to pressure which acts in a similar fashion to cooling and compresses the acyl chains of phospholipids while promoting a phase change from the liquid crystalline to the gel state (Mackey and Mañas 2008; Rivalain et al. 2010). Not only can pressure modify the fluidity and the permeability of the cell membrane (Hauben et al. 1997), but it can also alter the functioning of membrane-bound enzymes. Loss of the membrane integrity generally occurs at rather high pressure, between 1 and 2 kbar for the more sensitive exponentially growing cells (Pagán and Mackey 2000). Proteins are also sensitive to pressure, especially multimeric proteins, since their assembly is stabilized by weak bonds. In general, protein denaturation has a volume change of −10 to −100 mL mol^{-1} and occurs at pressures >4 kbar (Aertsen et al. 2009; Meersman and Heremans 2008). The reason for the decrease of volume during denaturation is not completely understood so far but involves electrostriction and the elimination of internal cavities present in the native conformation. Dissociations of multimeric proteins into single units have larger negative volume changes and occur at lower pressures (2–3 kbar) (Silva and Weber 1993). Because they retain most of their secondary structure and adopt a "molten globule-like structure," pressure-denatured proteins are actually quite different from temperature-denatured proteins (Meersman and Heremans 2008). The pressure–temperature phase diagram of proteins exhibits an elliptic curve (Hawley 1971; Suzuki 1960), which means that a pressure increase can either stabilize or destabilize protein structures depending on the starting value and on temperature (Fig. 3). Remarkably, the

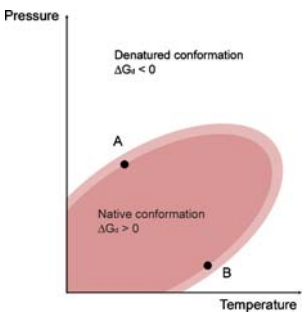

Fig. 3 Pressure–temperature diagram representing the elliptical stability domain of proteins based on Balny et al. (1997) and Eisenmenger and Reyes-De-Corcuera (2009). The regions inside and outside of the ellipses correspond to native and denatured conformations, respectively, and the intermediate region to a zone of reversible denaturation where the change of free Gibbs enthalpy upon denaturation (ΔG_d)=0. Depending on the initial conditions of pressure and temperature, an increase of pressure can have either a destabilizing effect (*A*) or a stabilizing effect (*B*) on the protein structure

pressure–temperature stability diagram of some bacteria such as *Escherichia coli* exhibits a similar elliptic shape as well, and therefore, it was proposed that proteins, and not cell membranes or nucleic acids, were responsible for the inactivation of microorganisms by pressure (Rivalain et al. 2010; Smeller 2002).

Cellular processes

Mesophilic bacteria such as *E. coli* are able to grow at high pressures of up to ~500 bar (ZoBell and Johnson 1949), which implies that the essential cellular processes are still functional under these conditions. The arrest of cellular processes is generally due to the dissociation or the change of conformation of the protein-based machineries. An example is protein synthesis that stops at ~700 bar because of the dissociation of ribosomes (Gross et al. 1993; Schulz et al. 1976). Ribosomes have a large negative volume change (at least −240 mL mol^{-1}) which makes them the most pressure-sensitive element of protein synthesis (Gross et al. 1993; Schulz et al. 1976). However, their dissociation is reversible below 1 kbar, and protein synthesis can resume once the pressure is released (Mackey and Mañas 2008). The RNA polymerase is more stable than ribosomes and dissociates into its subunits at 1.4 kbar for *E. coli* (Kawano et al. 2004). Nevertheless, RNA transcription starts to be affected at 200 bar already and is completely inhibited at 800 bar, probably because of conformational changes (Yayanos and Pollard 1969). DNA synthesis and chromosome replication are both very sensitive to pressure, especially chromosome replication which is inhibited at 500 bar (Bartlett 2002). Despite their great stability under pressure, DNA can undergo indirect damages as a result of endonuclease activation (Chilton et al. 1997). These endonucleases generate double-strand breaks in DNA that give the signal for inducing an SOS response in *E. coli* (Aertsen and Michiels 2005; Aertsen et al. 2004). Cell division is also sensitive to high pressure and stops before biomass production does, which can result in filamentous growth (ZoBell and Cobet 1964). Another well-known phenotype of high-pressure stress is the loss of motility (Meganathan and Marquis 1973), possibly due to perturbations of the cell membrane and flagellum apparatus.

Enzymatic reactions

As stated previously, pressure can accelerate or decelerate a reaction depending on the activation volume $V^{\neq}$, but it can also affect an enzymatic reaction rate by modifying the enzyme conformation and, for instance, its affinity for a substrate. Advantage can be taken of the increased thermostability of some enzymes under high pressure: Higher temperatures can be used to enhance the reaction rate because pressure helps in maintaining a functional structure (Eisenmenger and Reyes-De-Corcuera 2009). A comprehensive list of enzymes enhanced by high pressure was established by Eisenmenger and Reyes-De-Corcuera, among which various oxidoreductases and hydrolases relevant for food processing can be found (Eisenmenger and Reyes-De-Corcuera 2009).

Gene expression

In order to cope with the stress induced by high pressure, microorganisms have developed responses which share similarities with both cold-shock and heat-shock responses. Indeed, 4 cold-shock proteins and 11 heat-shock proteins were found to be transiently up-regulated in *E. coli* cells cultivated for 60 to 90 min at 550 bar (Welch et al. 1993), and this simultaneous expression of cold-shock and heat-shock proteins was later confirmed by genome-wide transcription studies (Ishii et al. 2005). Induction of apparently antagonist responses may seem at first contradictory, but these two classes of proteins actually have complementary functions: The cold-shock proteins help in maintaining membrane fluidity and guarantee an accurate protein translation, whereas heat-shock proteins deal with the refolding or degradation of denatured proteins (Aertsen and Michiels 2008; Arsène et al. 2000; Thieringer et al. 1998).

As can be expected, *rpoS*, the general stress response regulator in *E. coli*, plays a major role at high pressure (Aertsen and Michiels 2005; Mackey and Mañas 2008; Malone et al. 2006; Robey et al. 2001), and the periplasmic stress response regulator *rpoE* is up-regulated under these conditions as well (Malone et al. 2006). More surprising are the induction of oxidative stress at high pressure (Aertsen and Michiels 2005), the requirement of intact thioredoxin activity to cope efficiently with pressure (Malone et al. 2006), and the better pressure resistance of mutants defective in the assembly of iron–sulfur clusters (Malone et al. 2006). To explain these phenomena, Malone et al. proposed that (1) pressure denatures proteins in a way that exposes sulfhydryl groups and disulfide bridges to catalytic agents, thereby affecting the redox balance, and (2) it releases iron from Fe–S clusters, thus promoting Fenton reactions and the formation of reactive oxygen species (Malone et al. 2006). Furthermore, Aertsen et al. suggested that the inactivation of *E. coli* at high pressure might be the consequence of a suicide mechanism involving an oxidative burst caused by an imbalanced metabolism, an excess of reducing power, and derailing electron transfer reactions (Aertsen and Michiels 2005). The SOS stress response, which triggers the production of DNA repair proteins following DNA damage, was also found to be induced by high pressure (Aertsen et al. 2004; Bowman et al. 2008). SOS induction was shown to be at least partially responsible for the arrest of cell division and the observed filamentous growth under high pressure since the SOS protein SulA inhibits FtsZ-dependent ring formation

and, as a result, the initiation of septum formation (Aertsen and Michiels 2008; Huisman et al. 1984; Mukherjee et al. 1998). Interestingly, an *E. coli* strain harboring an *hns* deletion exhibited great pressure sensitivity, suggesting that the DNA-binding regulatory protein HN-S may be directly or indirectly involved in high-pressure response regulation (Ishii et al. 2005).

The cell membrane also seems particularly sensitive to pressure since the proportion of unsaturated fatty acids in the lipid bilayer was reported to increase with pressure (DeLong and Yayanos 1985). The production of several membrane proteins, such as the outer membrane proteins OmpH and OmpL (Bartlett et al. 1989; Welch and Bartlett 1996), several transporters (Ishii et al. 2005), and even terminal oxidases (Qureshi et al. 1998a; Qureshi et al. 1998b), was shown to vary with pressure in piezophilic microorganisms. Lastly, induction of genes associated with flagella assembly, chemotaxis, as well as lipid and peptidoglycan biosynthetic pathways was also revealed by transcriptome analyses of *Listeria monocytogenes* exposed to pressures of 4 and 6 kbar, whereas many genes involved in energy production and conversion, carbohydrate metabolism, and virulence were repressed (Bowman et al. 2008).

Effects of elevated pressure on bacteria (1–10 bar)

Cellular components and cellular processes

A direct effect of pressures below 10 bar on cellular components and cellular processes can reasonably be ruled out since destabilization starts under pressures at least 20 times larger (see previous discussion). This is supported by the fact that except for a small decrease in viability, no alteration of cell physiology (biomass production, carbon and nitrogen yield, respiratory quotient) was detected in *Pseudomonas putida* KT2440 cultivated at 7 bar (Follonier et al. 2012). Moreover, elevated pressures up to 11 bar have been successfully applied to cultivations with several yeasts and bacteria (see the following discussion). Nevertheless, indirect effects linked to the increase of gas solubility are likely to occur at elevated pressures. The occurrence of large dissolved oxygen tension (DOT) can indeed generate oxidative stress and result in the intracellular accumulation of reactive oxygen species (ROS) causing damages to RNA, DNA, proteins, and lipids (Cabiscol et al. 2000). Above a certain level, dissolved carbon dioxide tension (DCT) may also (1) affect the function of biological membranes, thus interfering with cell division, substrate uptake, and transport, (2) acidify the internal pH, (3) affect carboxylation/decarboxylation reactions, (4) alter the physico-chemical properties of enzymes and thereby their function, and (5) regulate virulence and toxin production in several pathogens (Dixon and Kell 1989; Stretton and Goodman 1998; Stretton et al. 1996). These effects were suggested to explain the growth inhibition observed at elevated DCT for various microorganisms (reviewed by Dixon and Kell (1989)).

Biotechnological applications based on high pressure

Food processing

Pathogen inactivation has been the main target of high-pressure processes, and research has been focused primarily on food products. It took about one century after Roger's discovery that high pressures were able to inactivate bacteria (Roger 1895) and Hite's suggestion that this could be applied to new food preservation techniques (Hite 1899) until the first implementation of high-pressure processes in the food industry. Pressure-based food processing has been recently reviewed in detail (Demazeau and Rivalain 2011; Heinz and Buckow 2010; Yaldagard et al. 2008; Zhang et al. 2011), and it will therefore only be briefly commented here. Moreover, for a list of the food pathogens (bacteria, viruses, and bacteriophages) that can be efficiently inactivated by high-pressure treatment, the reader is referred to the publication of Black et al. (2011). Today, 60 companies all over the world are commercializing more than 250 high-pressure-treated food products of which about one third corresponds to vegetable products (mainly avocado), about one third to meat products, and the last third to juices, seafood, fishes, and other products (Tonello 2011). Although about five times more expensive than processes based on heat treatment (Yaldagard et al. 2008), high-pressure processes have the advantage of preserving the nutritional and organoleptic properties of the food (taste, smell, appearance, texture) while inactivating pathogens. Indeed, since high pressure does not damage covalent bonds, it has a much smaller impact on vitamins and flavor molecules than temperature, and the processed food maintains fresh-like properties. Nevertheless, alterations may indirectly occur because of the enhancement/retardation of chemical or enzymatic reactions at high pressure, and this can have either positive or negative effects on the food stability (Oey et al. 2008a; Oey et al. 2008b; Sancho et al. 1999). For instance, the enhanced activity of lipoxygenase at high pressure is not desired because this enzyme is responsible for the degradation of chlorophyll and off-flavor development in frozen vegetables (Tedjo et al. 2000). In contrast, the enhanced activity of naringinase that hydrolyzes the bitter flavonone glycoside naringin (Ferreira et al. 2008) can be used to reduce the bitterness of grapefruit juices. Alteration of protein structures or modification of enzymatic reaction rates at high pressure can have further beneficial effects on food such as increasing the digestibility of milk proteins (Zeece et al. 2008), reducing allergic reactions against bovine gamma globulin (Yamamoto et al. 2010), and decreasing the viscosity of juices (Sila et al. 2007).

Disinfection of biomaterials

In the last decade, there has been growing interest for high-pressure disinfection processes of biomaterials such as bones, cartilages, and tendons. High-pressure treatment constitutes a valid alternative to gamma ray irradiation, heat treatment, and chemical inactivation that tend to deteriorate the biomechanical properties of the material. As a matter of fact, high pressure was shown not to alter the biomechanical and immunohistochemical properties of the material while being able to inactivate diverse viruses, bacteria, and fungi (Brouillet et al. 2009; Diehl et al. 2005; Diehl et al. 2006; Gollwitzer et al. 2009; Naal et al. 2008). Nevertheless, Gollitzer et al. showed that bacteria embedded in bones were less prone to high pressure inactivation than bacteria in suspension. Therefore, the establishment of efficient high-pressure disinfection methods of such systems still requires further research.

High-pressure treatments can also be used for the ex vivo devitalization of tumor-bearing bone segments before their implantation as an autograft. Indeed, normal eukaryotic cells and malignant cells, which are more sensitive to pressure than bacteria, are irreversibly damaged following high-pressure treatment, while the biomechanical properties of the biomaterial remain intact (Diehl et al. 2008). Moreover, the successful revitalization of high-pressure-treated bone segments has been demonstrated recently (Schauwecker et al. 2011), which augurs a successful implementation of this technique in the future.

Development of vaccines

The potential of high-pressure treatment for the production of vaccines has been known since 50 years (Basset et al. 1956), but only since the 1990s has research focused on it. The rationale behind the use of high pressure for vaccine production is that it would inactivate pathogens while keeping intact the interactions and structures necessary to induce an immune response. To date, high-pressure-based vaccines have been studied for a dozen of pathogens (reviewed by Shearer and Kniel (2009)), ranging from the yellow fever virus (Gaspar et al. 2008) to the poliovirus (Ferreira et al. 2009), the bacterium *Leptospira interrogans* serovar *hardjo* (Silva et al. 2001), the chicken parasite *Eimeria acervulina* (Shearer et al. 2007), and even mammalian tumor cells (Weiss et al. 2010).

Dissociation of amyloid fibrils

Recently, the use of high pressure for dissociating amyloid fibrils has been investigated (Foguel et al. 2003; Meersman et al. 2006; Torrent et al. 2006). Amyloid fibrils are fibrous protein aggregates characterized by their β-sheet structures and found in various debilitating diseases such as Alzheimer's disease, Parkinson's disease, and bovine spongiform encephalopathy. In the latter case, amyloid fibrils are formed from the scrapie isoform of a prion protein (PrPSc), which is derived from the cellular isoform (PrPC), and act as the infectious entity (Collinge and Clarke 2007). Remarkably, the extremely heat-resistant prion amyloid fibrils were shown to be less infective and more prone to proteinase K degradation after being simultaneously treated with high pressure (> 5 kbar) and high temperature (60 °C) (Fernandez Garcia et al. 2004). In addition, applying short high-pressure pulses (6.9–12 kbar at 121–137 °C) to prion infected meat was demonstrated to reduce considerably prion infectivity (Brown et al. 2003). More information about the mechanism of the amyloid fibril alteration under high pressure and the concomitant decrease of cytotoxicity can be found in the recent study of El Moustaine et al. (2011).

Protein refolding

High pressure of slightly lower range (~1–3 kbar) has been investigated since the last decade for dissolving protein aggregates and refolding proteins to their native state. The formation of protein aggregates, also called inclusion bodies, is one of the biggest problems encountered in the production of recombinant proteins. Indeed, biological activity requires soluble and correctly folded proteins. Applying high pressure to protein aggregates can simultaneously dissociate inclusion bodies and refold misfolded proteins, which is clearly more efficient and time saving than the traditional method consisting first in solubilizing the aggregates in a concentrated chaotrope solution and then in refolding the denatured proteins by decreasing the chaotrope concentration via dialysis, diafiltration, or dilution (Crisman and Randolph 2009). Moreover, the pressure-based procedure can, in most cases, be applied independently of protein concentration and requires little or no chaotropic agents. To date, about 20 proteins have been successfully refolded using high pressure (Arana et al. 2010; Balduino et al. 2010; Chura-Chambi et al. 2008; Fradkin et al. 2010; Fraga et al. 2010; John et al. 2002; Lee et al. 2006; Lefebvre et al. 2004; Malavasi et al. 2011; Schoner et al. 2005; Seefeldt et al. 2007; Seefeldt et al. 2004; St. John et al. 2001; St. John et al. 1999; Torrent et al. 2003), and high pressure was, in some cases, the only method allowing the recovery of fully active proteins. In addition, over a hundred therapeutic proteins were claimed to have been successfully refolded by pressure in a commercial setting (Qoronfleh et al. 2007).

Biotechnological applications based on elevated pressure

Enhancement of the OTR for high-cell-density cultivations

One of the major limiting factors of high-cell-density cultivations with microorganisms is the oxygen supply. If the

oxygen supply is insufficient, cell growth is prevented, and the cell metabolism may also be affected and result, for instance, in an enhancement of the production of unwanted by-products such as acetate or ethanol. Among the various methods studied for enhancing the OTR, applying elevated pressure in the range of 1 to 11 bar constitutes a relatively novel, yet simple method. Bioprocesses under elevated pressure have been investigated with various microorganisms, including yeasts and bacteria (*Absidia coerula*, *Arxula adeninivorans*, *Aureobasidium pullulans*, *Corynebacterium glutamicum*, *E. coli*, *Kluyveromyces marxianus*, *P. putida*, *Saccharomyces cerevisiae*, and *Yarrowia lipolytica*) (Aguedo et al. 2005; Belo et al. 2003; Dufresne et al. 1990; Follonier et al. 2012; Jia and Cui 2009; Knabben et al. 2010a; Knabben et al. 2010b; Knoll et al. 2007; Knoll et al. 2005; Lopes et al. 2008; Lopes et al. 2009; Ma et al. 2010; Matsui et al. 2006; Noger et al. 2006; Pinheiro et al. 2000; Yang and Wang 1992). In general, applying elevated pressure resulted in larger cell and product productivities (Table 1), except in some cases where growth inhibition occurred due to large DCT (Knoll et al. 2005; Matsui et al. 2006). Since the microorganisms and their ability to synthesize a product can exhibit different sensitivities to increased DOT or DCT (Belo et al. 2003), the effect of pressure on product formation must be evaluated on a case-by-case basis. For instance, the production of decalactone was apparently quite sensitive to the changes of DOT occurring upon pressure increase (Aguedo et al. 2005), which implies that for this process, it is crucial to increase pressure in parallel with the oxygen demand to avoid the formation of large DOT. Moreover, it is, in general, recommended to apply such a careful process control in order to prevent oxidative stress that may alter not only the product synthesis but also the cell physiology (Belo et al. 2003; Follonier et al. 2012).

Elevated pressure, oxygen-enriched air, and reduced feeding rate were compared by Ma et al. (2010) in fed-batch processes with *E. coli* producing human-like collagen. The highest cell density and the best volumetric product yield were achieved with elevated pressure, while the production of acetate (by-product) and the increase in broth viscosity were the largest with oxygen-enriched air, possibly because of cell lysis.

Bioprocesses that are supposed to be carried out under elevated pressure (up to approximately 10 bar) require special equipment (bioreactor, valves, pipes, pumps, aeration system) capable to work efficiently under these conditions and have therefore higher investment costs. Maier reported that the cost of bioreactors smaller than 40 m^3 was not influenced by the operating pressure up to 6 bar, but that it would increase by about 10% per bar at higher pressures (Maier 2002). Also, pressure generators more powerful and thus more expensive than the usual ones may be needed above 5 bar. Nevertheless, this constitutes a one-time expense, while the operating costs of bioprocesses at elevated pressure are essentially the same as at normal pressure. As a result, using elevated pressure instead of oxygen-enriched air can become much more profitable in the long run, the continuous use of pure oxygen being extremely expensive. Lastly, Knoll et al. showed that the energy efficiency and the cost efficiency of the oxygen transfer rate, which depend on the agitation power, the aeration rate, and the operating pressure, could be enhanced by at least 13% when using elevated pressure instead of ambient pressure (Knoll et al. 2005). This means that in addition to the benefits arising from larger volumetric productivities, applying pressure to bioprocesses may as well reduce to some extent the fermentation costs.

Reduction of explosion risks in two-liquid phase cultivations with flammable organic solvents

Microorganisms such as pseudomonads are able to grow on aliphatic and aromatic hydrocarbons and can be used as

Table 1 Major improvements related to biomass and/or product synthesis observed when applying elevated pressure during bioprocesses

Strain	Improvements upon pressure	Reference
E. coli	Two-fold increase in cell productivity by increasing the pressure to 11 bar	(Knoll et al. 2007)
	1.7- and 2-fold increase in cell density and product yield, respectively, by increasing the pressure to 0.6 bar	(Ma et al. 2010)
A. adeninivorans	Maximum cell density (225 gL^{-1}) ever achieved for this strain by increasing the pressure to 5 bar	(Knoll et al. 2007)
A. pullulans	1.5- and 1.3-fold increase in biomass and product (pullulan) final concentrations at 6.2 bar	(Dufresne et al. 1990)
S. cerevisiae	1.8- and 2-fold increase in biomass yield and biomass productivity, respectively, by increasing the pressure to 10 bar	(Belo et al. 2003)
C. glutamicum	Maximum cell density (226 gL^{-1}) ever achieved for this strain by increasing the pressure to 10 bar	(Knoll et al. 2007)
Y. lipolytica	10-fold increase in lipase specific activity at 8 bar	(Lopes et al. 2008)
	5-fold and 3.4 fold increase in biomass production and specific growth rate, respectively, at 6 bar	(Lopes et al. 2009)

biocatalysts for their oxidation. These bioprocesses are generally performed in two-liquid phase systems where the cells grow in the aqueous medium and the hydrophobic oxidation substrate is dissolved in a second organic solvent phase (Schmid et al. 1998). However, the combination of aerobic operating conditions and flammable solvents presents an explosion hazard that requires special safety measurements. One strategy that was analyzed by Schmid et al. for the safe operation of such bioprocesses consisted in applying elevated pressure (Schmid et al. 1999). Indeed, by working at conditions above the critical pressure and below the critical temperature, the vapor pressure of the flammable solvent could stay below the explosion limit. For instance, a pressure higher than 4.9 bar at 30 °C would be sufficient to prevent the formation of explosive atmospheres during bioprocesses with octane (Schmid et al. 1999).

Conclusive remarks

Although most of the research about pressure in biotechnology has been focused on the development of food processing methods—which led to the successful implementation of various industrial processes—a number of studies performed during these last decades have demonstrated a much broader scope of application for both high and elevated pressure. Technologies for the refolding of aggregated proteins in their native form are already commercially available, but it will certainly take some more time until pressure-based disinfection methods of biomaterials or vaccines will reach a similar level of maturity. As a matter of fact, strict regulations apply to medical products and technologies, and the certification procedures can be very difficult and time consuming. However, the early studies seem promising, and it can reasonably be expected that pressure-based disinfection methods will be approved in the future. Besides, the application of elevated pressure in the range of 1–10 bar for bioprocesses is attracting more and more interest due to its large potential for enhancing process productivities and its low cost in the long run compared to the use of oxygen-enriched air. While further research is still needed in this field, there have been an increasing number of studies for the last 20 years demonstrating the potential of this method.

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