

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

Stéphanie Follonier · Sven Panke · Manfred Zinn

Received: 9 November 2011 / Revised: 17 December 2011 / Accepted: 19 December 2011 / Published online: 31 January 2012
© Springer-Verlag 2012

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

S. Follonier · M. Zinn
Laboratory for Biomaterials, Empa—Swiss Federal
Laboratories for Materials Science and Technology,
Lerchenfeldstrasse 5,
9014 St. Gallen, Switzerland

S. Follonier
e-mail: stephanie.follonier@hevs.ch

S. Panke
Bioprocess Laboratory, Department of Biosystems
Science and Engineering (D-BSSE), ETH Zurich,
Mattenstrasse 26,
4058 Basel, Switzerland
e-mail: sven.panke@bsse.ethz.ch

M. Zinn (✉)
Institute of Life Technologies, HES-SO Valais -
University of Applied Sciences Western Switzerland,
Rue du Rawyl 47,
1950 Sion, Switzerland
e-mail: manfred.zinn@hevs.ch

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.

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

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 ΔV^\ddagger (Eq. 2)

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

Increasing the pressure can thus either accelerate or decelerate reactions depending on the sign of the activation volume ΔV^\ddagger . 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

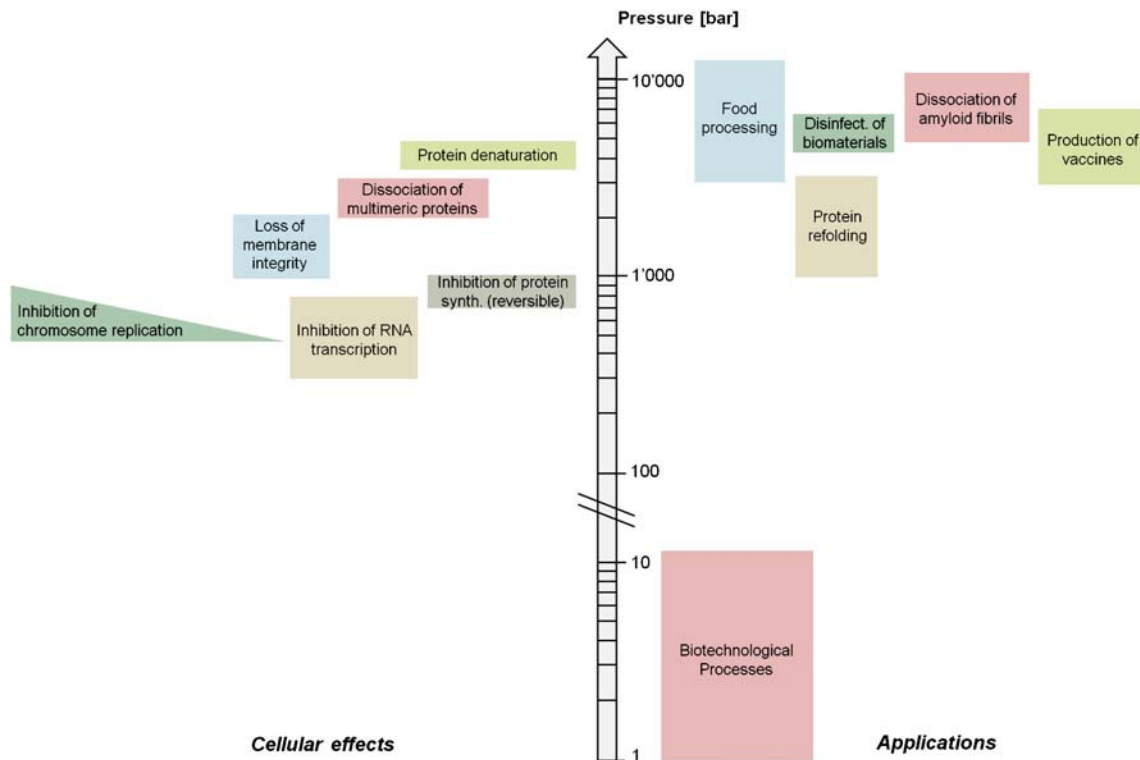
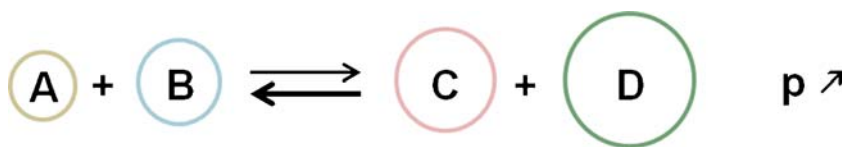


Fig. 1 Overview on the effects of pressures in the range of 1 bar to 10 kbar on microorganisms (*left*) and on their major applications in biotechnology (*right*). The range of pressure corresponding to each

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 O₂ and CO₂ (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).

$$p_g = K_H \cdot c_g. \quad (3)$$

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 degassed 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

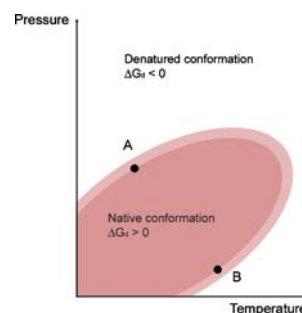


Fig. 3 Pressure–temperature diagram representing the elliptical stability domain of proteins based on Balny et al. (1997) and Eisenmenger and Reyes-De-Corcuera (2009). The regions inside and outside of the ellipses correspond to native and denatured conformations, respectively, and the intermediate region 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^\ddagger , 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 (Cabisco 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 lipoxigenase 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, Gollwitzer 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 (PrP^{Sc}), which is derived from the cellular isoform (PrP^C), 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 (Qoronfle 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 coerulea*, *Arxula adenivorans*, *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 m³ 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
<i>E. coli</i>	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)
<i>A. adenivorans</i>	Maximum cell density (225 gL ⁻¹) ever achieved for this strain by increasing the pressure to 5 bar	(Knoll et al. 2007)
<i>A. pullulans</i>	1.5- and 1.3-fold increase in biomass and product (pullulan) final concentrations at 6.2 bar	(Dufresne et al. 1990)
<i>S. cerevisiae</i>	1.8- and 2-fold increase in biomass yield and biomass productivity, respectively, by increasing the pressure to 10 bar	(Belo et al. 2003)
<i>C. glutamicum</i>	Maximum cell density (226 gL ⁻¹) ever achieved for this strain by increasing the pressure to 10 bar	(Knoll et al. 2007)
<i>Y. lipolytica</i>	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.

Acknowledgments This work was supported by the Swiss National Science Foundation (SNF), grant 315200-116812/1.

References

- Aertsen A, Michiels CW (2005) Mrr instigates the SOS response after high pressure stress in *Escherichia coli*. *Mol Microbiol* 58(5):1381–1391
- Aertsen A, Michiels C (2008) Cellular impact of sublethal pressures on *Escherichia coli*. In: Michiels C, Bartlett DH, Aertsen A (eds) High-pressure microbiology. ASM Press, Washington DC, pp 87–100
- Aertsen A, Van Houdt R, Vanoirbeek K, Michiels CW (2004) An SOS response induced by high pressure in *Escherichia coli*. *J Bacteriol* 186(18):6133–6141
- Aertsen A, Meersman F, Hendrickx MEG, Vogel RF, Michiels CW (2009) Biotechnology under high pressure: applications and implications. *Trends Biotechnol* 27(7):434–441
- Aguedo M, Gomes N, Garcia E, Waché Y, Mota M, Teixeira JA, Belo I (2005) Decalactone production by *Yarrowia lipolytica* under increased O₂ transfer rates. *Biotechnol Lett* 27(20):1617–1621
- Arana ME, Powell GK, Edwards LL, Kunkel TA, Petrovich RM (2010) Refolding active human DNA polymerase γ from inclusion bodies. *Protein Expr Purif* 70(2):163–171
- Arsène F, Tomoyasu T, Bukau B (2000) The heat shock response of *Escherichia coli*. *Int J Food Microbiol* 55(1–3):3–9
- Balduino K, Spencer P, Malavasi N, Chura-Chambi R, Lemke L, Morganti L (2010) Refolding by high pressure of a toxin containing seven disulfide bonds: bothropstoxin-1 from *Bothrops jararacussu*. *Mol Biotechnol*:1–7
- Balny C, Mozhaev VV, Lange R (1997) Hydrostatic pressure and proteins: basic concepts and new data. *Comp Biochem Physiol* 116(4):299–304
- Bartlett DH (2002) Pressure effects on *in vivo* microbial processes. *Biochim Biophys Acta-Protein Struct Mol Enzymol* 1595(1–2):367–381
- Bartlett D, Wright M, Yayanos AA, Silverman M (1989) Isolation of a gene regulated by hydrostatic pressure in a deep-sea bacterium. *Nature* 342(6249):572–574
- Basset J, Lépine P, Chaumont L (1956) Effet des hautes pressions sur le virus de la poliomyélite (souche Lansing). *Ann Inst Pasteur (Paris)* 90(5):575–593
- Belo I, Pinheiro R, Mota M (2003) Fed-batch cultivation of *Saccharomyces cerevisiae* in a hyperbaric bioreactor. *Biotechnol Progr* 19(2):665–671
- Black EP, Stewart CM, Hoover DG (2011) Microbial aspects of high-pressure food processing. In: Zhang HQ, Barbosa-Cánovas GV, Balasubramaniam VM, Dunne CP, Farkas DF, Yuan JTC (ed) Nonthermal processing technologies for food, IFT Press and Wiley-Blackwell, pp 51–71.
- Bowman JP, Bittencourt CR, Ross T (2008) Differential gene expression of *Listeria monocytogenes* during high hydrostatic pressure processing. *Microbiology* 154(2):462–475
- Brouillet M, Gautier H, Miègeville A-F, Bouler J-M, Merle C, Caillon J (2009) Inactivation of *Staphylococcus aureus* in calcium phosphate biomaterials via isostatic compression. *J Biomed Mater Res B Appl Biomater* 91B(1):348–353
- Brown P, Meyer R, Cardone F, Pocchiarri M (2003) Ultra-high-pressure inactivation of prion infectivity in processed meat: a practical method to prevent human infection. *Proc Natl Acad Sci USA* 100(10):6093–6097
- Cabiscol E, Tamarit J, Ros J (2000) Oxidative stress in bacteria and protein damage by reactive oxygen species. *Int Microbiol* 3(1):3–8
- Chilton P, Isaacs NS, Mackey B, Stenning R (1997) The effects of high hydrostatic pressure on bacteria. In: Heremans K (ed) High pressure research in the biosciences and biotechnology. Leuven University Press, Leuven, pp 225–228
- Chura-Chambi RM, Genova LA, Affonso R, Morganti L (2008) Refolding of endostatin from inclusion bodies using high hydrostatic pressure. *Anal Biochem* 379(1):32–39
- Collinge J, Clarke AR (2007) A general model of prion strains and their pathogenicity. *Science* 318(5852):930–936
- Crisman RL, Randolph TW (2009) Refolding of proteins from inclusion bodies is favored by a diminished hydrophobic effect at elevated pressures. *Biotechnol Bioeng* 102(2):483–492

- DeLong E, Yayanos A (1985) Adaptation of the membrane lipids of a deep-sea bacterium to changes in hydrostatic pressure. *Science* 228(4703):1101–1103
- Demazeau G, Rivalain N (2011) The development of high hydrostatic pressure processes as an alternative to other pathogen reduction methods. *J Appl Microbiol* 110(6):1359–1369
- Diehl P, Schmitt M, Schauwecker J, Eichelberg K, Gollwitzer H, Gradinger R, Goebel M, Preissner KT, Mittelmeier W, Magdolen U (2005) Effect of high hydrostatic pressure on biological properties of extracellular bone matrix proteins. *Int J Mol Med* 16(2):285–289
- Diehl P, Steinhäuser E, Gollwitzer H, Heister C, Schauwecker J, Milz S, Mittelmeier W, Schmitt M (2006) Biomechanical and immunohistochemical analysis of high hydrostatic pressure-treated Achilles tendons. *J Orthop Sci* 11(4):380–385
- Diehl P, Schauwecker J, Mittelmeier W, Schmitt M (2008) High hydrostatic pressure, a novel approach in orthopedic surgical oncology to disinfect bone, tendons and cartilage. *Anticancer Res* 28(6B):3877–3883
- Dixon NM, Kell DB (1989) The inhibition by CO₂ of the growth and metabolism of micro-organisms. *J Appl Bacteriol* 67(2):109–136
- Dufresne R, Thibault J, Leduy A, Lencki R (1990) The effects of pressure on the growth of *Aureobasidium pullulans* and the synthesis of pullulan. *Appl Microbiol Biotechnol* 32(5):526–532
- Eisenmenger MJ, Reyes-De-Corcuera JI (2009) High pressure enhancement of enzymes: a review. *Enzyme Microb Technol* 45(5):331–347
- El Moustaine D, Perrier V, Acquattella-Tran Van Ba I, Meersman F, Ostapchenko VG, Baskakov IV, Lange R, Torrent J (2011) Amyloid features and neuronal toxicity of mature prion fibrils are highly sensitive to high pressure. *J Biol Chem* 286(15):13448–13459
- Fang J, Zhang L, Bazylinski DA (2010) Deep-sea piezosphere and piezophiles: geomicrobiology and biogeochemistry. *Trends Microbiol* 18(9):413–422
- Fernandez Garcia A, Heindl P, Voigt H, Buttner M, Wienhold D, Butz P, Starke J, Tauscher B, Pfaff E (2004) Reduced proteinase K resistance and infectivity of prions after pressure treatment at 60 °C. *J Gen Virol* 85(1):261–264
- Ferreira L, Afonso C, Vila-Real H, Alfaia A, Ribeiro MHL (2008) Evaluation of the effect of high pressure on naringin hydrolysis in grapefruit juice with naringinase immobilised in calcium alginate beads. *Food Technol Biotechnol* 46(2):146–150
- Ferreira E, Mendes YS, Silva JL, Galler R, Oliveira AC, Freire MS, Gaspar LP (2009) Effects of hydrostatic pressure on the stability and thermostability of poliovirus: a new method for vaccine preservation. *Vaccine* 27(39):5332–5337
- Foguel D, Suarez MC, Ferrão-Gonzales AD, Porto TCR, Palmieri L, Einsiedler CM, Andrade LR, Lashuel HA, Lansbury PT, Kelly JW, Silva JL (2003) Dissociation of amyloid fibrils of α -synuclein and transthyretin by pressure reveals their reversible nature and the formation of water-excluded cavities. *Proc Natl Acad Sci USA* 100(17):9831–9836
- Follonier S, Henes B, Panke S, Zinn M (2012) Putting cells under pressure: a simple and efficient way to enhance the productivity of medium-chain-length polyhydroxyalkanoate in processes with *Pseudomonas putida* KT2440. *Biotechnol Bioeng* 109(2):451–461
- Fradkin AH, Boand CS, Eisenberg SP, Rosendahl MS, Randolph TW (2010) Recombinant murine growth hormone from *E. coli* inclusion bodies: expression, high-pressure solubilization and refolding, and characterization of activity and structure. *Biotechnol Progr* 26(3):743–749
- Fraga TR, Chura-Chambi RM, Gonçalves AP, Morais ZM, Vasconcellos SA, Morganti L, Martins EAL (2010) Refolding of the recombinant protein OmpA70 from *Leptospira interrogans* from inclusion bodies using high hydrostatic pressure and partial characterization of its immunological properties. *J Biotechnol* 148(2–3):156–162
- Gaspar LP, Mendes YS, Yamamura AMY, Almeida LFC, Caride E, Gonçalves RB, Silva JL, Oliveira AC, Galler R, Freire MS (2008) Pressure-inactivated yellow fever 17DD virus: implications for vaccine development. *J Virol Meth* 150(1–2):57–62
- Gollwitzer H, Mittelmeier W, Brendle M, Weber P, Miethke T, Hofmann GO, Gerdesmeyer L, Schauwecker J, Diehl P (2009) High hydrostatic pressure for disinfection of bone grafts and biomaterials: an experimental study. *Open Orthop J* 3:1–7
- Gross M, Lehle K, Jaenicke R, Nierhaus KH (1993) Pressure-induced dissociation of ribosomes and elongation cycle intermediates. *Eur J Biochem* 218(2):463–468
- Hauben KJA, Bartlett DH, Soontjens CCF, Cornelis K, Wuytack EY, Michiels CW (1997) *Escherichia coli* mutants resistant to inactivation by high hydrostatic pressure. *Appl Environ Microbiol* 63(3):945–950
- Hawley SA (1971) Reversible pressure–temperature denaturation of chymotrypsinogen. *Biochemistry* 10(13):2436–2442
- Heinz V, Buckow R (2010) Food preservation by high pressure. *J Verbr Lebensm* 5(1):73–81
- Hite BH (1899) The effect of pressure in the preservation of milk. *W Va Univ Agr Exp Sta Bull.* 58.
- Huisman O, D’Ari R, Gottesman S (1984) Cell-division control in *Escherichia coli*: specific induction of the SOS function SfiA protein is sufficient to block septation. *Proc Natl Acad Sci USA* 81(14):4490–4494
- Ishii A, Oshima T, Sato T, Nakasone K, Mori H, Kato C (2005) Analysis of hydrostatic pressure effects on transcription in *Escherichia coli* by DNA microarray procedure. *Extremophiles* 9(1):65–73
- Jia SR, Cui JD (2009) Production of hydrocortisone by *Absidia coerulea* in moderate pressure bioconversion system. *Korean J Chem Eng* 26(4):1084–1089
- John RJS, Carpenter JF, Randolph TW (2002) High-pressure refolding of disulfide-cross-linked lysozyme aggregates: thermodynamics and optimization. *Biotechnol Progr* 18(3):565–571
- Kawano H, Nakasone K, Matsumoto M, Yoshida Y, Usami R, Kato C, Abe F (2004) Differential pressure resistance in the activity of RNA polymerase isolated from *Shewanella violacea* and *Escherichia coli*. *Extremophiles* 8(5):367–375
- Knabben I, Regestein L, Grumbach C, Steinbusch S, Kunze G, Büchs J (2010a) Online determination of viable biomass up to very high cell densities in *Arxula adenivorans* fermentations using an impedance signal. *J Biotechnol* 149(1–2):60–66
- Knabben I, Regestein L, Marquering F, Steinbusch S, Lara AR, Büchs J (2010b) High cell-density processes in batch mode of a genetically engineered *Escherichia coli* strain with minimized overflow metabolism using a pressurized bioreactor. *J Biotechnol* 150(1):73–79
- Knoll A, Maier B, Tscherrig H, Büchs J (2005) The oxygen mass transfer, carbon dioxide inhibition, heat removal, and the energy and cost efficiencies of high pressure fermentation. *Adv Biochem Eng Biotechnol* 92:77–99
- Knoll A, Bartsch S, Husemann B, Engel P, Schroer K, Ribeiro B, Stockmann C, Seletzky J, Büchs J (2007) High cell density cultivation of recombinant yeasts and bacteria under non-pressurized and pressurized conditions in stirred tank bioreactors. *J Biotechnol* 132(2):167–179
- Lee S-H, Carpenter JF, Chang BS, Randolph TW, Kim Y-S (2006) Effects of solutes on solubilization and refolding of proteins from inclusion bodies with high hydrostatic pressure. *Protein Sci* 15(2):304–313
- Lefebvre BG, Gage MJ, Robinson AS (2004) Maximizing recovery of native protein from aggregates by optimizing pressure treatment. *Biotechnol Progr* 20(2):623–629
- Lopes M, Gomes N, Gonçalves C, Coelho MAZ, Mota M, Belo I (2008) *Yarrowia lipolytica* lipase production enhanced by increased air pressure. *Lett Appl Microbiol* 46(2):255–260

- Lopes M, Gomes N, Mota M, Belo I (2009) *Yarrowia lipolytica* growth under increased air pressure: influence on enzyme production. *Appl Biochem Biotechnol* 159(1):46–53
- Ma X, Fan D, L-a S, Cai Q, Chi L, Zhu C, Mi Y, Y-e L (2010) Oxygen transfer rate control in the production of human-like collagen by recombinant *Escherichia coli*. *Biotechnol Appl Biochem* 55(4):169–174
- Macgregor RBJ (2002) The interactions of nucleic acids at elevated hydrostatic pressure. *Biochim Biophys Acta Protein Struct Mol Enzymol* 1595(1–2):266–276
- Mackey B, Mañas P (2008) Inactivation of *Escherichia coli* by high pressure. In: Michiels C, Bartlett DH, Aertsen A (eds) High-pressure microbiology. ASM Press, Washington DC, pp 53–85
- Maier B (2002). Auslegung und Betrieb von Rührkesselreaktoren unter erhöhtem Druck. Dissertation, Rheinisch-Westfälische Technische Hochschule Aachen.
- Malavasi NV, Foguel D, Bonafe CFS, Braga CACA, Chura-Chambi RM, Vieira JM, Morganti L (2011) Protein refolding at high pressure: optimization using eGFP as a model. *Proc Biochem* 46(2):512–518
- Malone AS, Chung YK, Yousef AE (2006) Genes of *Escherichia coli* O157:H7 that are involved in high-pressure resistance. *Appl Environ Microbiol* 72(4):2661–2671
- Matsui T, Shinzato N, Yokota H, Takahashi J, Sato S (2006) High cell density cultivation of recombinant *E. coli* with a pressurized culture. *Proc Biochem* 41(4):920–924
- Meersman F, Heremans K (2008) High hydrostatic pressure effects in the biosphere: from molecules to microbiology. In: Michiels C, Bartlett DH, Aertsen A (eds) High-pressure microbiology. ASM Press, Washington DC, pp 1–17
- Meersman F, Dobson CM, Heremans K (2006) Protein unfolding, amyloid fibril formation and configurational energy landscapes under high pressure conditions. *Chem Soc Rev* 35(10):908–917
- Meganathan R, Marquis RE (1973) Loss of bacterial motility under pressure. *Nature* 246(5434):525–527
- Mozhaev VV, Heremans K, Frank J, Masson P, Balny C (1996) High pressure effects on protein structure and function. *Protein Struct Funct Bioinf* 24(1):81–91
- Mukherjee A, Cao C, Lutkenhaus J (1998) Inhibition of FtsZ polymerization by SulA, an inhibitor of septation in *Escherichia coli*. *Proc Natl Acad Sci USA* 95(6):2885–2890
- Naal FD, Schauwecker J, Steinhäuser E, Milz S, von Knoch F, Mittelmeier W, Diehl P (2008) Biomechanical and immunohistochemical properties of meniscal cartilage after high hydrostatic pressure treatment. *J Biomed Mater Res B Appl Biomater* 87B(1):19–25
- Noger D, Hartmann R, Zinn M (2006) Fermentation von Biopolyester (PHA) im Hochdruck-Bioreaktor. *Chem Ing Tech* 78(9):1381–1381
- Oey I, Lille M, Van Loey A, Hendrickx M (2008a) Effect of high-pressure processing on colour, texture and flavour of fruit- and vegetable-based food products: a review. *Trends Food Sci Tech* 19(6):320–328
- Oey I, Van der Plancken I, Van Loey A, Hendrickx M (2008b) Does high pressure processing influence nutritional aspects of plant based food systems? *Trends Food Sci Tech* 19(6):300–308
- Oger PM, Jebbar M (2010) The many ways of coping with pressure. *Res Microbiol* 161(10):799–809
- Pagán R, Mackey B (2000) Relationship between membrane damage and cell death in pressure-treated *Escherichia coli* cells: differences between exponential- and stationary-phase cells and variation among strains. *Appl Environ Microbiol* 66(7):2829–2834
- Pinheiro R, Belo I, Mota M (2000) Air pressure effects on biomass yield of two different *Kluyveromyces* strains. *Enzym Microb Tech* 26(9–10):756–762
- Qoronfle MW, Hesterberg LK, Seefeldt MB (2007) Confronting high-throughput protein refolding using high pressure and solution screens. *Protein Expr Purif* 55(2):209–224
- Qureshi MH, Kato C, Horikoshi K (1998a) Purification of a *ccb*-type quinol oxidase specifically induced in a deep-sea barophilic bacterium, *Shewanella* sp. strain DB-172 F. *Extremophiles* 2(2):93–99
- Qureshi MH, Kato C, Horikoshi K (1998b) Purification of two pressure-regulated c-type cytochromes from a deep-sea barophilic bacterium, *Shewanella* sp. strain DB-172 F. *FEMS Microbiol Lett* 161(2):301–309
- Rivalain N, Roquain J, Demazeau G (2010) Development of high hydrostatic pressure in biosciences: pressure effect on biological structures and potential applications in biotechnologies. *Biotechnol Adv* 28(6):659–672
- Robey M, Benito A, Hutson RH, Pascual C, Park SF, Mackey BM (2001) Variation in resistance to high hydrostatic pressure and *rpoS* heterogeneity in natural isolates of *Escherichia coli* O157:H7. *Appl Environ Microbiol* 67(10):4901–4907
- Roger H (1895) Action des hautes pressions sur quelques bactéries. *Arch Physiol Norm Path* 7:12–17
- Sancho F, Lambert Y, Demazeau G, Largeteau A, Bouvier J-M, Narbonne J-F (1999) Effect of ultra-high hydrostatic pressure on hydrosoluble vitamins. *J Food Eng* 39(3):247–253
- Schauwecker J, Von Eisenhart-Rothe R, Burgkart R, Tuebel J, Schmitt M, Mittelmeier W, Diehl P (2011) Revitalization of human bone after extracorporeal high hydrostatic pressure treatment. *Anticancer Res* 31(4):1235–1239
- Schmid A, Kollmer A, Mathys RG, Witholt B (1998) Developments toward large-scale bacterial bioprocesses in the presence of bulk amounts of organic solvents. *Extremophiles* 2(3):249–256
- Schmid A, Kollmer A, Sonnleitner B, Witholt B (1999) Development of equipment and procedures for the safe operation of aerobic bacterial bioprocesses in the presence of bulk amounts of flammable organic solvents. *Bioprocess Eng* 20(2):91–100
- Schoner BE, Bramlett KS, Guo H, Burris TP (2005) Reconstitution of functional nuclear receptor proteins using high pressure refolding. *Mol Genet Metab* 85(4):318–322
- Schulz E, Lüdemann HD, Jaenicke R (1976) High pressure equilibrium studies on the dissociation-association of *E. coli* ribosomes. *FEBS Lett* 64(1):40–43
- Schumpe A, Quicker G, Deckwer W-D (1982) Gas solubilities in microbial culture media. Heidelberg, Springer Berlin, pp 1–38
- Seefeldt MB, Ouyang J, Froland WA, Carpenter JF, Randolph TW (2004) High-pressure refolding of bikunin: efficacy and thermodynamics. *Protein Sci* 13(10):2639–2650
- Seefeldt MB, Crouch C, Kendrick B, Randolph TW (2007) Specific volume and adiabatic compressibility measurements of native and aggregated recombinant human interleukin-1 receptor antagonist: density differences enable pressure-modulated refolding. *Biotechnol Bioeng* 98(2):476–485
- Shearer AEH, Kniel KE (2009) High hydrostatic pressure for development of vaccines. *J Food Protect* 72(7):1500–1508
- Shearer AEH, Wilkins GC, Jenkins MC, Kniel KE (2007) Effects of high hydrostatic pressure on *Eimeria acervulina* pathogenicity, immunogenicity and structural integrity. *Innovat Food Sci Emerg Tech* 8(2):259–268
- Sila DN, Smout C, Satara Y, Truong V, Loey AV, Hendrickx M (2007) Combined thermal and high pressure effect on carrot pectinmethylesterase stability and catalytic activity. *J Food Eng* 78(3):755–764
- Silva JL, Weber G (1993) Pressure stability of proteins. *Annu Rev Phys Chem* 44(1):89–113
- Silva CCM, Giongo V, Simpson AJG, Camargos ERdS, Silva JL, Koury MC (2001) Effects of hydrostatic pressure on the *Leptospira interrogans*: high immunogenicity of the pressure-inactivated serovar *hardjo*. *Vaccine* 19(11–12):1511–1514
- Smeller L (2002) Pressure-temperature phase diagrams of biomolecules. *Biochim Biophys Acta Protein Struct Mol Enzymol* 1595(1–2):11–29

- St. John RJ, Carpenter JF, Randolph TW (1999) High pressure fosters protein refolding from aggregates at high concentrations. *Proc Natl Acad Sci USA* 96(23):13029–13033
- St. John RJ, Carpenter JF, Balny C, Randolph TW (2001) High pressure refolding of recombinant human growth hormone from insoluble aggregates. *J Biol Chem* 276(50):46856–46863
- Stretton S, Goodman AE (1998) Carbon dioxide as a regulator of gene expression in microorganisms. *Antonie Leeuwenhoek* 73(1):79–85
- Stretton S, Marshall KC, Dawes IW, Goodman AE (1996) Characterisation of carbon dioxide-inducible genes of the marine bacterium, *Pseudomonas sp.* S91. *FEMS Microbiol Lett* 140(1):37–42
- Suzuki K (1960) Studies on the kinetics of protein denaturation under high pressure. *Rev Phys Chem Jpn* 29(2):91–98
- Tedjo W, Eshtiaghi MN, Knorr D (2000) Impact of supercritical carbon dioxide and high pressure on lipoxygenase and peroxidase activity. *J Food Sci* 65(8):1284–1287
- Thieringer HA, Jones PG, Inouye M (1998) Cold shock and adaptation. *Bioessays* 20(1):49–57
- Tonello C (2011) Case studies on high-pressure processing of foods. In: Zhang HQ, Barbosa-Cánovas GV, Balasubramaniam VM, Dunne CP, Farkas DF, Yuan JTC (ed) *Nonthermal processing technologies for food*, IFT Press and Wiley-Blackwell, pp 36–50.
- Torrent J, Alvarez-Martinez MT, Heitz F, Liautard J-P, Balny C, Lange R (2003) Alternative prion structural changes revealed by high pressure. *Biochemistry* 42(5):1318–1325
- Torrent J, Balny C, Lange R (2006) High pressure modulates amyloid formation. *Protein Pep Lett* 13:271–277
- Weiss EM, Meister S, Janko C, Ebel N, Schlücker E, Meyer-Pittroff R, Fietkau R, Herrmann M, Gaipl US, Frey B (2010) High hydrostatic pressure treatment generates inactivated mammalian tumor cells with immunogenic features. *J Immunot* 7(3):194–204
- Welch T, Bartlett D (1996) Isolation and characterization of the structural gene for OmpL, a pressure-regulated porin-like protein from the deep-sea bacterium *Photobacterium* species strain SS9. *J Bacteriol* 178(16):5027–5031
- Welch TJ, Farewell A, Neidhardt FC, Bartlett DH (1993) Stress-response of *Escherichia coli* to elevated hydrostatic pressure. *J Bacteriol* 175(22):7170–7177
- Yaldagard M, Mortazavi SA, Tabatabaei F (2008) The principles of ultra high pressure technology and its application in food processing/preservation: a review of microbiological and quality aspects. *Afr J Biotechnol* 7(16):2739–2767
- Yamamoto S, Mikami N, Matsuno M, Hara T, Odani S, Suzuki A, Nishiumi T (2010) Effects of a high-pressure treatment on bovine gamma globulin and its reduction in allergenicity. *Biosci Biotechnol Biochem* 74(3):525–530
- Yang J-D, Wang NS (1992) Oxygen mass transfer enhancement via fermentor headspace pressurization. *Biotechnol Prog* 8:244–251
- Yayanos AA, Pollard EC (1969) A study of the effects of hydrostatic pressure on macromolecular synthesis in *Escherichia coli*. *Biophys J* 9(12):1464–1482
- Zeece M, Huppertz T, Kelly A (2008) Effect of high-pressure treatment on *in-vitro* digestibility of β -lactoglobulin. *Innovat Food Sci Emerg Tech* 9(1):62–69
- Zhang HQ, Barbosa-Cánovas GV, Balasubramaniam VM, Dunne CP, Farkas DF, Yuan JTC (2011) *Nonthermal processing for food* IFT Press and Wiley-Blackwell.
- ZoBell CE, Cobet AB (1964) Filament formation by *Escherichia coli* at increased hydrostatic pressures. *J Bacteriol* 87(3):710–719
- ZoBell CE, Johnson FH (1949) The influence of hydrostatic pressure on the growth and viability of terrestrial and marine bacteria. *J Bacteriol* 57(2):179–189