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Review

Sterilization and virus inactivation by supercritical fluids (a review)

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ABSTRACT

While supercritical processes are developing both for "classical" applications in food industry and in new domains related to Health Sciences, the interactions of supercritical fluids (SCFs) with living microorganisms are of growing importance. It is known for long that supercritical fluid extraction processes do protect the processed materials from oxidation and contamination with organic solvents and prevent bio-burden increase. Moreover, SCFs were also shown to have the ability to kill most microorganisms and to "inactivate viruses", including human pathogenic strains. This paper intends to summarize the present state-of-the-art in order to underline the promising future of SCF sterilization/pasteurization and virus inactivation as an alternative "green" method to classical processes that cannot be used in a growing number of cases: thermolabile products degrading by heat sterilization, or compounds reacting with sterilizing chemicals (hydrogen peroxide, ethylene oxide, peracetic acid, etc.), or radiolysis of biomolecules during irradiation. Process implementation and commercial development are then discussed in light of future challenges in terms of regulatory, economical and environment requirements.

Contents

1. Introduction

For millenaries, food preservation has been fundamental for human kind as it has conditioned its survival and expansion under all climates. During the recent decades, pasteurization and sterilization have been a fast growing activity, especially for food preservation, medical devices and pharmaceuticals. Meanwhile the classical processes using heat cannot be used for heat-sensitive products, most operators have been more and more reluctant to move to low temperature processes based on irradiation and chemicals (like H_2O_2 or ethylene oxide) for many reasons including cost, safety and environmental concerns.

Living organisms are sensitive to their environment in which they can maintain metabolic activity within narrow limits of

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temperature, pH, hydrostatic pressure, and chemical composition, although a large variety of single cell organisms are capable to grow under extreme environmental conditions (i.e. deep-sea thermophilic bacteria).

For long, high pressure treatment is used for pest control and sterilization in food industries – especially in Japan where irradiation was never accepted – as alternative to heat treatment that generally degrades product quality (aspect, taste, vitamin content, etc.). However, the required hydrostatic pressure for efficient sterilization is extremely high (4000–8000 bar) and exposure times are also considerable, which leads to costs incompatible with most markets.

As mentioned in early publications and patents [1–13], it is known for 20 years or more that supercritical fluid exposure can be considered as a less expensive variant through various processes working at much lower pressures, where the product is contacted with carbon dioxide, possibly added with water or ethanol or other additives like acetic acid or hydrogen peroxide.

More recently, much attention has been paid to supercritical fluid pasteurization of food products and sterilization of various products and items, with a special attention dedicated to inactivation of spores that are known to be highly resistant to heat, radiation, and chemical agents. In addition, few investigators worked on virus inactivation in plasma fractions and implants.

As many studies were published during the last decades, it seems now timely to gather all these data – that are sometimes contradictory – and to evaluate what are the main challenges to solve to reach commercial development and acceptance of this "green" technology in regards to the regulatory, economical and environment requirements. This paper clearly intends to widespread this knowledge towards the scientists belonging to the supercritical fluid "community" that may not be aware of SCFs potential for sterilization.

2. Definitions

For clarity, it seems necessary to list some basic definitions and common practices according to international standards:

- *Sterility*: Sterility is the absence of viable microorganisms. As sterility cannot be guaranteed by testing; it has to be assured by the application of a suitably validated production process according to protocols defined by control authorities.
- *Sterilization:* The act of rendering something free from living cells, either by removing, killing or inactivating all microorganisms, including vegetative forms and spores.
- *Validation:* In order to validate a sterilization process, standardized preparations of selected microorganisms (called biological indicators) are used. They usually consist of a population of bacterial spores. The recommended species by the European and the US Pharmacopeia are *Geobacillus stearothermophilus* for steam or gas sterilization, *Bacillus subtilis* for dry-heat or gas sterilization, *Bacillus pumilus* for irradiation and *Pseudomonas diminuta* for sterile filtration.
- *Survival ratio, reduction factor and sterilization efficacy:* The sterilization efficacy is often defined from the survival ratio of the number of viable microorganisms after the sterilization (N) to the number before processing (N_0), and expressed in form of the reduction factor or degree of inactivation (DI):

$$\mathrm{DI} = -\log_{10}\frac{N}{N_0} \tag{1}$$

The higher is this number, the higher is the process efficacy.

- *Sterilization kinetics:* For a given process operated in given conditions, changes in microbial populations versus time is commonly described by the survivor curve equation:

$$\log_{10}\frac{N}{N_0} = \frac{-t}{D} \tag{2}$$

where D is the decimal reduction time, or time required for a $1-\log$ reduction in the microbial population, by analogy with the first-order kinetic model for chemical reactions. Alternative models are being developed to explain microbial inactivation kinetics when the linearity of the data is questionable.
- *Sterility assurance level:* SAL is the probability of a non-sterile item in a population. The SAL of a process for a given product is established by appropriate validation studies. A SAL value of 10^{-6} is generally regarded as acceptable.
- *Pasteurization:* This word refers to a moderate heat treatment, invented by Pasteur, leading to microorganisms inactivation without significant product degradation, essentially used on food products. By extension, pasteurization is also used to designate other processes applicable to food products (such as CO_2 treatment). The difference between sterilization and pasteurization is that the latter does not kill spores.

3. Biological effects of supercritical fluids on microorganisms

3.1. Early work

Early work showed that gaseous CO_2 and N_2O, even at low pressure (below critical pressure), inhibit the growth [1,2] and boost the inactivation rate of microorganisms including spores during irradiation [3] or thermal treatment. Heat treatment at 50–55 °C in the presence of CO_2 at 6 bar has the same lethal effect on several bacteria, fungi and yeasts as heat treatment at 60–65 °C in presence of air, or, in other words, operating with this gas pressure could reduce by 50% the time of pasteurization at a given temperature [14].

3.2. Vegetative microorganisms

From several early sources [5,6,8,13,15], comparison of the survival curves of microorganisms in contact with a pressurised gas like nitrogen, ethane or propane, and with a sub-/super-critical fluid (carbon dioxide, ethane, propane), clearly demonstrates that the bactericidal effect of these fluids cannot be attributed to hydrostatic pressure in the range of tens or hundreds of bars, but to specific interactions depending on fluid chemical nature, nitrogen being almost inactive while CO_2, N_2O and propane are very efficient in cell inactivation. On the other hand, for long, it has been recognized that gaseous CO_2 can inhibit microbial growth [1,2,4], leading to its use in the preservation of packed foods, although its inactivation effect seems reversible. Even at pressure as low as 6 bar, this gas exhibits a significant bactericide or bacteriostatic effect [14]. Moreover, this specific effect is definitely supported by the comparison of cell number decay of various microorganisms when submitted to a very high hydrostatic pressure with and without carbon dioxide [17]. For example, the decay of *Escherichia coli* in CO_2 at 150 bar and 35 °C during 15 min was similar to the one observed at 3000 bar at ambient temperature during the same period of time [17].

So, there is no doubt that this bactericidal effect is caused by specific interactions between the living cell and the fluid that readily dissolves inside the cell. As clearly shown by several authors [18–20], cell decay is considerably increased when CO_2 pressure is raised beyond the critical pressure, boosting both fluid dissolution inside cell and membrane lipids interaction. A similar conclusion was raised with propane [16]. As discussed in depth by