Sterilization by supercritical CO_2: FASTECO_2 project

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1. Introduction

The field of implantable medical devices (IMD) is expanding and requires alternative to conventional sterilization methods which could degrade the IMD or may leave residual traces of toxic compounds. The use of supercritical CO_2 ($scCO_2$) seems to be an interesting new way to sterilize IMD: it is nontoxic, abundant and can be used on heat sensitive materials. In this respect, the French project FASTECO_2 began in January 2020 with the collaboration of two academic partners (Aix Marseille University and the University of Lille) and two industrial partners (Cousin Surgery and Lattice Medical). The main objective of this project is to show the technical and industrial feasibility of the IMD sterilization by $scCO_2$. To achieve this goal, it is required to determine processing conditions which allow a total inactivation of microorganisms (6-log reduction) according to the ISO 14937:2009 standards. Due to their high resistance, spores are the reference to test the efficiency of a sterilization method[1]. We have started our experiments using the spore forming bacterium *Bacillus subtilis* as dry inoculum to fit to IMD conditions. It would appear that only one other study used $scCO_2$ dry process to inactivate *B. subtilis*. They obtained total inactivation with additive addition and pressure cycles[2]. In our work, in order to have conditions which respect the IMD, we decided to use $ScCO_2$ in batch processing without additive and to vary three parameters: temperature, pressure and contact time between the scCO2 and the bacteria spores.

2. Materials and Methods

B. subtilis spore suspensions ($\approx 10^9$ UFC·mL^{-1}) are spread on a glass slide which is placed in the autoclave. The CO_2 is cooled at -10 °C and injected in the autoclave until the defined pressure is reached. The desired temperature is maintained in the autoclave thanks to the circulation of heated water into a double walled envelop (Figure 1). The depressurization is realized with a flow rate of 7.6 bar·h^{-1}. The bacterial reduction (R) is defined from the ratio of the number of viable organisms before (N_0) and after (N) sterilization with the equation : $R = log_{10}\frac{N}{N_0}$.

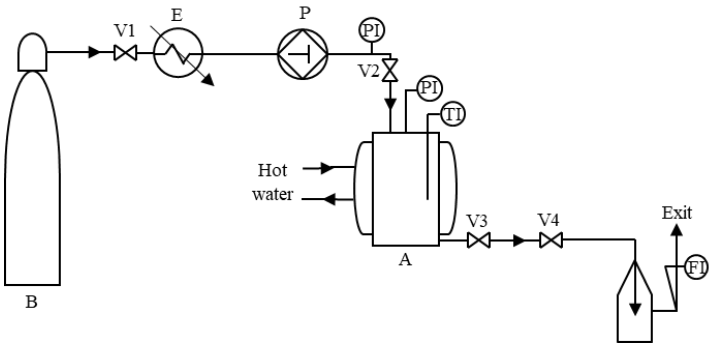

Figure 1. Process diagram of $scCO_2$ sterilization. B: CO_2 pressurized bottle; V1, V2 and V3: valve; V4: micrometric valve; E: cooler; P: CO_2 high pressure liquid pump; A: sterilization autoclave.

3. Results and discussion

First assays of sterilization with $scCO_2$ were performed at a temperature of 50 °C, contact time of 4 h and a pressure range from 80 to 300 bar. In these conditions, as expected, the value of the bacterial reduction decreases when the pressure increases. The bacterial reduction remains higher than -1 but lower than 0, far away from the goal of 6-log reduction (Figure 2).

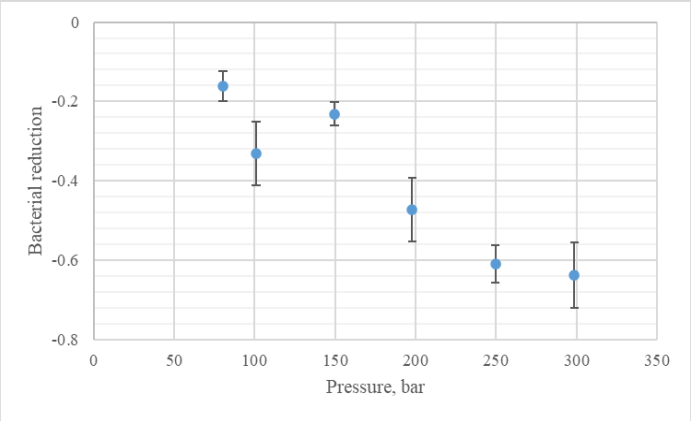

Figure 2. Effect of pressure on the inactivation of *Bacillus subtilis* in a dry inoculum using $scCO_2$ at temperature 50 °C and time 4 h.

4. Conclusions

Although our experimental conditions were not able to achieve a 6-log reduction, we consider that $scCO_2$ has an impact on bacterial spore viability. We are currently investigating new $scCO_2$ sterilization conditions, in particular with the use of additive which preserve the IMD properties, in order to reach the reduction goal. Inactivation kinetics studies are also in progress.

References

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2. M.A. da Silva, *J. Supercrit. Fluids*, **2016,** vol.109, p.87-94.