J Ind Microbiol Biotechnol (2011) 38:29–37
DOI 10.1007/s10295-010-0824-4

ORIGINAL PAPER

Human pathogens, nosocomial infections, heat-sensitive textile implants, and an innovative approach to deal with them

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Received: 26 March 2010 / Accepted: 26 July 2010 / Published online: 8 September 2010

Abstract Implantable polymers, as used for biomedical applications, inherently have to be sterile. Nonetheless, most implants, particularly those comprised of biomaterials developed in recent years for tissue engineering, are heat sensitive. Therefore, use of hazardous (radio)chemicals—due to lack of alternative methods—is still state of the art for sterilization processes. The drawbacks of these techniques, both drastic and well known, lead to the demand for an alternative sterilization method, which is equally obvious and urgent. High-pressure fluid treatment is a low-temperature technique that is already in use for pasteurization of liquid food products. This paper explores inactivation of vegetative microorganisms, spores, and endotoxins adherent to solid surfaces using compressed CO_2. Pressures ranging from 50 to 100 bar and temperatures from 25 °C to 50 °C were explored to investigate liquid, gaseous or supercritical state. Analysis of variance (ANOVA) and statistical modeling were used to identify the optimum parameter settings for inactivation of pathogenic bacteria and fungi (*Candida albicans*, *Staphylococcus aureus*). The addition of small amounts of ozone ensures inactivation of persistent spores (*Bacillus stearothermophilus*, *B. subtilis*) up to 10^6 cfu/ml, while endotoxins remain in practically unchanged concentration on the polymer surface. We then discuss environmental issues of the process and inactivation mechanisms. The replacement of conventional chemicals with nonpersistent ones resolves organizational and safety-related issues and protects natural resources as well as handling staff. The pressurized-fluid-based method exhibits mild treatment parameters, thus protecting sensitive textures. Finally, an outlook on possible applications of this innovative technique is presented.

Keywords Sterilization · Spores · Supercritical fluid · Ozone · Biopolymer

Introduction

In daily life, microorganisms are ubiquitous. Few of them are pathogens, causing infections. Fungi, molds, vegetative bacteria, and their persistent forms (spores) along with viruses and endotoxins represent possible threats to the human body. There are significant concerns in various industries involved in food processing and packaging, and healthcare regarding how to deal with microbial contamination. In the medical environment the need for proper disinfection of medical equipment such as workwear and other fabrics that come into contact with the patient is fundamental. Nosocomial infections are those related to a stay in hospital, and are regarded as one of the most important types [39]. Moreover, the expansion of the field of tissue engineering has created a broad necessity for mild sterilization methods for biomaterials.

Conventional sterilization processes have several limitations, which become particularly clear in polymer treatments: thermal methods, such as autoclaving, damage the structure and reduce the product lifetime of heat-sensitive

This article is part of the BioMicroWorld 2009 Special Issue.

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material, requiring an alternative low-temperature method. However, textural properties of complex implants with numerous and nonhomogeneous cavities make it difficult to use liquid- or plasma-based methods [28]. Penetration into three-dimensional materials (textiles, meshes, scaffolds), containing small pores or constrictions at points of intersection of threads, must be ensured. Preservation of physical properties and inertness of the treated material are basic requirements which cannot be met by ionizing irradiation in γ- or β-ray sterilization, since these methods often lead to formation of persistent radicals and residues that cause allergic reactions, inflammation or hemolysis [16, 33]. The problem concerning sterilization by gaseous chemical means (e.g., ethylene oxide) is threefold: firstly, diffusion of the applied chemical is typically very slow, increasing treatment time; secondly, residual chemicals can be harmful to patient health and can cause irritation or even hemolysis [13]; as a result, some implants cannot be treated chemically; finally, most chemical sterilants are flammable and/or toxic and constitute a significant danger to staff health and the environment [29].

Carbon dioxide is nonflammable, nonpolar, inert, readily available and, in the supercritical state, has favorable transport and pore-diffusion properties due to its low viscosity [4]. It can therefore accelerate processes which are limited by mass-transfer velocity or which have to overcome the obstacle of surface tension. Its use can speed up reactions and mass transport due to the lack of phase interfaces, probably also affecting the protective cell layers of microbes. A promising alternative inactivation technique is therefore high-pressure (HP) CO_2 treatment between 50 and 500 bar, which is already in use for pasteurization of liquid food products [43]. Generally, industrial-scale plants using highly compressed CO_2 are well established for treatment of coffee and tea, and extraction of aromatic compounds and other nutritional products. HP dry cleaning using CO_2 has become well known in recent years, and several industrial-scale plants are operational in the USA and Europe [2, 3]. The technique allows for mild treatment parameters, thus protecting product texture. Accordingly, dense gaseous, liquid or supercritical carbon dioxide ($scCO_2$) ($T > 31°C$, $P > 74$ bar) is generally suitable, but has not been investigated in detail for (bio)polymer sterilization yet [36].

A few groups have reported use of compressed fluids for enhancing the effect of a sterilizing agent. It has been shown that applying pure supercritical fluid (SCF) results in lower inactivation of vegetative bacteria than using SCF with dissolved water [11]. Little research has been done on additives such as acetic acid [27], triclosan or propanol [37] for CO_2-based sterilization. Matthews' group focused on implant treatment and achieved promising results, particularly using H_2O_2 in trace amounts [26]. Further work, concentrating on ethanol and hydrogen peroxide, has been compiled in a review [25]. Effectiveness apparently correlates with—and is thus limited by—the solubility of the additive in CO_2.

This paper in contrast deals with applying a gas—namely ozone—as additive in HP CO_2, overcoming the drawback of the dissolution limit. In such a process, production of ozone can be carried out on-site by corona discharge and, after use, unreacted ozone can be converted to oxygen by catalytic reaction. Besides, while the great majority of former evaluations concerning HP CO_2 inactivation focused on microbes in solution, the present work deals with treatment of microbes on solid surfaces.

The first task of this investigation is to verify the efficacy of an innovative process for inactivating relevant surface concentrations of nosocomial vegetative germs on medical polymers by applying HP CO_2. At the same time, the factors that influence the efficacy of this process are identified and their significance quantified by statistical means [design of experiments (DoE) and ANOVA]. We also discuss the solvent properties that CO_2 has on the bacterial cell wall, thus contributing to inactivation. Secondly, the study tests whether complete sterilization of implantable polymers can be achieved by mixing additives into CO_2. Our working hypothesis is that the inactivating effect of ozone can be enhanced by use of compressed (gaseous, liquid or SCF) CO_2. Since we are aware of only one further study on HP inactivation when the fluid had to pass through a packaging barrier [45], the third task is to show sterilization of prepacked material. Beside microorganisms, also subcellular systems constitute a possible danger for human health. The final challenge of this study is therefore to investigate whether a sufficiently low level of endotoxins (lipopolysaccharides, LPS) can be achieved for safe implantation using (combinations of) highly pressurized fluids.

Materials and methods

High-pressure CO_2 apparatus

A flowchart of the applied process is shown in Fig. 1. The CO_2 apparatus is described in more detail elsewhere [12] and was comprised of a cylindrical washing chamber (VE-2) with volume of 1.5 l and a perforated, rotating inner drum. Carbon dioxide of technical grade (Air Liquide, Germany) was supplied from a gas-bomb bundle VE-1, cooled (HE-1), and compressed by a HP pump (P-1) (Maximator, Germany). Cellulosic sample swatches contaminated with microorganisms (described below) were placed into the high-pressure chamber, and subsequently the chamber was filled with ozone derived on-site from oxygen via corona discharge from an ozonizer (E-3) Modular 16 HC (Wedeco, Germany).

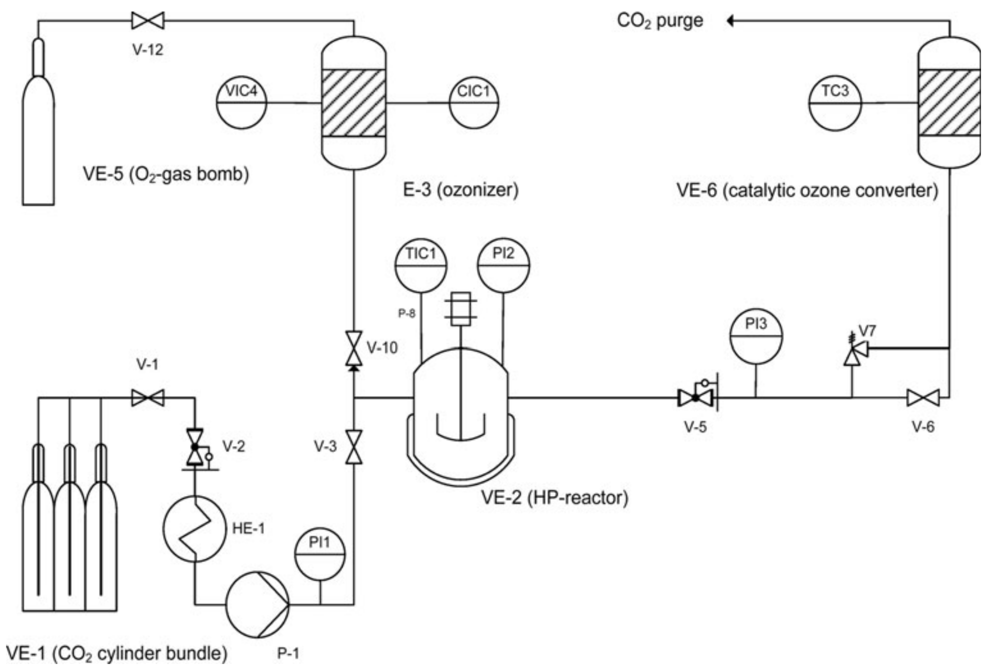

Fig. 1 Flowchart of the sterilization setup, showing main apparatus and additional information

The required pressure and temperature were adjusted, and continuously recorded. All experiments were carried out batchwise. After the respective treatment time, the pressure was released at constant flow of 75 g/min controlled with a mass-flow meter RHE 08 (Rheonik, Germany), and the treated samples were processed. The investigated CO_2 pressure ranged from 50 to 100 bar, and temperature was set to 20°C, 35°C, and 50°C. Elimination of ozone takes place on-site after the treatment using a catalytic converter (VE-6) CODw (Wedeco, Germany).

Sterility testing

Biomonitors of vegetative bacteria, yeasts, spores, and endotoxins were ordered from wfk Institute (Germany). We investigated Gram-positive bacterium *Staphylococcus aureus* (ATCC 6538) and yeast *Candida albicans* (ATCC 10231) on nitrocellulose membrane (5×40 mm^2) with bioburden of $(6.5 \pm 0.5) \times 10^6$ cfu/membrane without additional protein contamination. The microorganisms were dried for 3 h at 36°C (at 30°C for *C. albicans*) and packaged in SteriBags [commercially available packages with paper membrane and high-density polyethylene (HDPE) film; Megro GmbH & Co. KG, Germany]. After HP treatment the biomonitors were put into 10 ml casein-peptone soymeal-peptone (CASO) agar (for *C. albicans* malt extract; ME-Bouillon), resuspended, and plated on CASO agar or malt extract agar (MEA), respectively. CASO plates were incubated at 36°C, MEA plates at 30°C. Quantification of cfu was carried out after 48 h at 36 ± 1°C for *S. aureus* and 30 ± 1°C for *C. albicans*. Cultivation of the microorganisms followed German Society for Hygiene and Microbiology (*Deutsche Gesellschaft für Hygiene und Mikrobiologie*, DGHM) method no. 6 and manufacture and evaluation of biomonitors DGHM methods no. 17. Furthermore, spores of Gram-positive bacteria *B. atrophaeus* (ATCC 9372) and *B. stearothermophilus* (ATCC 7953) were used. The spores were fixed on nitrocellulose membranes (5×40 mm^2) with sample strips of 10^3, 10^4, 10^5, 10^6, and 10^7 cfu/carrier, purchased from HS System and Prozesstechnik GmbH (Germany) (residual moisture 5.4 ± 0.7% w/w). Treated biomonitors were resuspended in 10 ml trypton soja bouillon (TSB; Oxoid, The Netherlands) and incubated at 36°C (for *B. stearothermophilus* at 58°C). After incubation time of 14 days, growth was determined. All examinations included positive and negative control. All tests were carried out in three replicates.

A limulus amebocyte lysate (LAL) test for endotoxin inactivation was carried out on implantable meshes. Two different sample types were manufactured: The first kind was inoculated with 1 ml solution containing 10^5 EU/ml endotoxin from *Escherichia coli* 055:B5 and dried overnight at room temperature (1 EU = 1 endotoxin unit = 1 ng endotoxin). The second kind was incubated at 37°C in an *E. coli* broth and then dried for 3 h at the same temperature. Samples were packaged in SteriBags. For evaluation, kinetic turbidometric method was applied. It uses the fact that the time until a defined turbidity change of lysate of horseshoe crab lycocytes occurs is proportional to sample endotoxin concentration. Treated samples were put in 100 ml double-distilled (dd) water to resuspend remaining endotoxin. The test follows method C of *European Pharmacopeia* (chapter 2.6.14). Manufacture and testing of the samples were carried out by GFPS mbH (Germany).

Data and polymer analysis

Design of experiments (DoE) was used to investigate the significance of the varied parameters on the disinfection efficacy and the interactions between those parameters. The derived data were evaluated by multifactor analysis of variance (ANOVA) using Fisher's range test at error level of 0.05. For deriving a mathematic model (Eq. 1) of the process (N_0 and N are the bacterial concentrations before and after treatment), Minitab software (Minitab Corp., USA) was used. $x_{i,j}$ are the variables (parameters, e.g., pressure, temperature) $x_i x_j$ stand for interaction of these parameters, and $a_{i,j}$ are the constants that are determined experimentally using least-squares method.

$$\log\left(\frac{N_0}{N}\right) = a_0 + \sum_{i=1}^{k} a_i x_i + \sum_{i<j}^{k-1}\sum_{i<j}^{k} a_{ij} x_i x_j + \sum_{i=1}^{k} a_{ii} x_i^2. \qquad (1)$$

The pressure range in the DoE was chosen such that the effect of liquid, gaseous, and $scCO_2$ could be examined. Accordingly, the temperatures were selected, ensuring that the heat stress level of conventional sterilization processes was not reached by far. Treatment time was comparable to a steam-based process, being the reference technology in sterilization. Water addition was chosen so that the effect of saturated CO_2 could be investigated. Typical implantable fabrics (Dynamesh®), comprising, e.g., meshes and yarns made of polyvinylidene difluoride (PVDF) and polypropylene (PP), were provided by an industrial partner (FEG Textile Technology, Germany). Chemical properties as well as insights into surface morphology, topography, and properties down to atomic level of the treated polymers are described elsewhere [10, 12].

Results and discussion

A number of microorganisms, including bacteria, molds, and yeasts, can be inactivated or damaged by exposure to dense CO_2. In Fig. 2 we illustrate in a Pareto chart of effects the influencing factors for inactivation to compare the relative magnitude and statistical significance of both main and interaction effects. It becomes clear that no single mechanism of action is predominant; different microorganisms are damaged by supercritical fluid conditions for diverse reasons. Additionally, statistical analysis provides evidence for interacting mechanisms. The effects of process conditions are often confounded in literature: different process conditions including temperature, pressure, exposure time, and fluid shear forces have been employed and show interacting effects, but are rarely tested for significance. Therefore, design of commercial units requires careful consideration of the microorganisms targeted and the most effective process or optimum conditions for a sufficiently broad range of microorganisms.

The Pareto chart in Fig. 2a shows the factors in decreasing order of influence on inactivation of *S. aureus*. Parameters with experimental value $F_{exp.}$ higher than a theoretical value obtained from variance analysis $F_{th.}$ are significant, with significance level $\alpha = 0.05$. Pressure shows a clear influence on inactivation. Also ozone concentration was identified to be significant within the investigated range. Interacting parameters pressure/temperature (AD) indicate that probably also the phase (gaseous, liquid or supercritical) in which CO_2 is present in the process could play a vital role. The Pareto chart does not indicate yet whether the effect is positive or negative.

Furthermore, we show a significant difference in importance of the influencing factors between the bacteria and the yeast. For instance, in Fig. 2b, no significant interaction between the factors of pressure and ozone

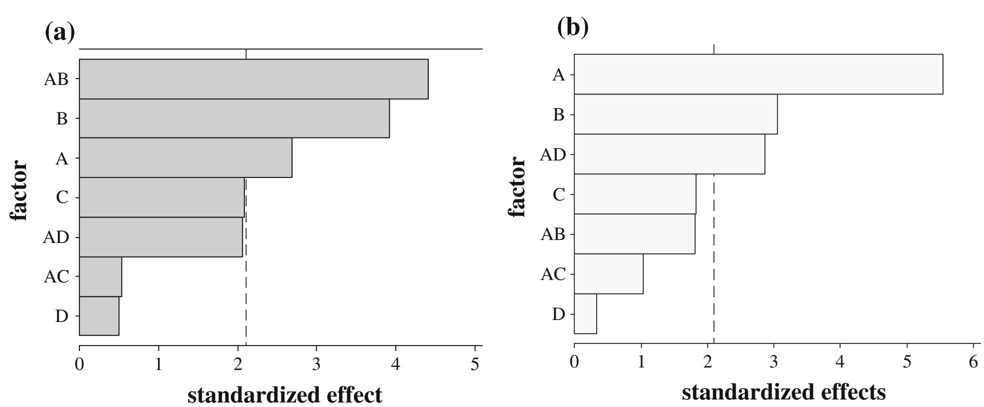

Fig. 2 Pareto-chart of standardized effects for the log-reduction of **a** *S. aureus* and **b** *C. albicans*: *A* = pressure, *B* = ozone concentration, *C* = time, *D* = temperature [significance threshold is given by the *broken line*: $F_{th.}$ = 2,101 (α = 0.05); any effect that extends past this *reference line* is significant]

concentration can be found, while Fig. 2a indicates that this interaction clearly influences inactivation. Both the CO_2 pressure applied as well as ozone have a significant effect on inactivation. The other factors were proven to have only insignificant effect within their investigated range. For further research this means that quantifying the influence of treatment time has to be investigated more closely below 20 min to understand the kinetics of inactivation, which could not be investigated in this work due to technical restrictions.

Table 1, on the other hand, demonstrates that a reduction of 5 log cycles of *C. albicans* is possible without addition of ozone, as long as working conditions are supercritical. At subcritical conditions, we observed higher standard deviations. Since the process conditions during all three replicated experiments were kept constant, we must conclude that those conditions lead to greater uncertainty of inactivation. We speculate that the supercritical fluid has a homogenizing effect. Contrarily, at subcritical conditions, the effect of ozone might be influenced by possible inhomogeneity in the bioindicator (microorganism layers or coagulations that have protecting properties).

A reduction of 6 log cycles was—at the treatment times applied—only possible with addition of 70 mg O_3/m^3. Results of inactivation of *S. aureus* differ in details from those of the *Candida* species. In addition, a secondary—but nonetheless meaningful—result is that the fluid (liquid, gaseous, and supercritical) is capable of passing through the package barrier.

Since *S. aureus* is the most important germ responsible for nosocomial infections, it is used as a reference in clinical studies and research work. A comparative investigation between vegetative germs including *Listeria*, *Bacillus*, *Salmonella*, and *Escherichia* [46] as well as *Saccharomyces* and *Pseudomonas* [14, 24, 31] in liquid media identified *Staphylococcus* as the most resistant germ against high-pressure inactivation. This statement seems to be too undifferentiated, taking the results of the present study into account. Reduction values differ only insignificantly for most parameter settings. For some settings—as can be seen from Table 1—bacterial inactivation is even slightly higher, rather indicating lower resistance against pressure treatment compared with the yeast.

We hypothesize that, beside the recently proven inactivation effect of pH drop [42], CO_2 could fluidize the cell wall (and maybe also the spore wall to a smaller extent), and thus enable a sterilizing agent to enter the cell/core. As solvent power varies with pressure [4] so does partition coefficient, which is key in inactivation of vegetative germs [22, 23]. Hydrophobicity of a compound, expressed as log *P* value, is correlated with the toxicity, and it is stated that log *P* between 1 and 5 can be regarded as toxic. For compressed CO_2, log *P* has been approximated by Nakagami et al. [32] to increase from 0.5 to 2.0 for a pressure increase from 50 to 150 bar, which is considered lipophilic [22], being comparable to the values of conventional solvents such as 1-hexanol (1.87), phenol (1.45), and 1-butanol (0.88). This is supported by Bothun et al. [5, 6], who studied the influence of pressurized fluids on bilayer fluidity of aqueous liposomal dipalmitoylphosphatidylcholine (DPPC), used as a model for cellular membrane. They demonstrated that DPPC bilayers show fluidization and melting-point depression upon pressurization with CO_2.

To summarize, it was clearly found that, for further experiments with spores, mainly investigation of pressure level and ozone concentration would be of interest. Treatment time was kept constant at 220 min and temperature at 35°C. The contour diagram in Fig. 3b illustrates inactivation of *B. stearothermophilus* spores depending on ozone concentration and applied CO_2 pressure. The area that indicates a reduction of more than 5.5 log cycles covers a wide range of pressure and concentration. Below 70 mg/m^3 ozone, insufficient spore inactivation could be achieved (<5.5 log cycles). The influence of pressure is apparently only minor; however, inactivation beyond a value of 5.3 log cycles is not possible using pure ozone. In contrast, Fig. 3a indicates that solely the addition of

Table 1 Inactivation level of *S. aureus* and *C. albicans* ($n = 3$) at varying conditions using the evaluation of the experimental plan from DoE (SD standard deviation)

Exp. no.	Pressure (bar)	Ozone (g/m^3)	Time (min)	Temp. (°C)	$\log(N_0/N)$ *S. aureus*		$\log(N_0/N)$ *C. albicans*	
					Mean	SD	Mean	SD
1	0	0	20	20	0.7	0.5	0.4	0.6
2	80	0	20	50	4.6	0.3	5.1	1.1
3	0	70	20	50	4.5	2.3	1.4	1.5
4	80	70	20	20	2.9	2.1	4.4	2.8
5	0	0	220	50	0.7	0.7	0.2	0.6
6	80	0	220	20	4.6	2.2	3.9	2.0
7	0	70	220	20	6.2	0.4	5.0	1.4
8	80	70	220	50	5.8	0.5	6.2	0.6
Central point	40	35	120	35	6.0	0.7	5.3	1.1

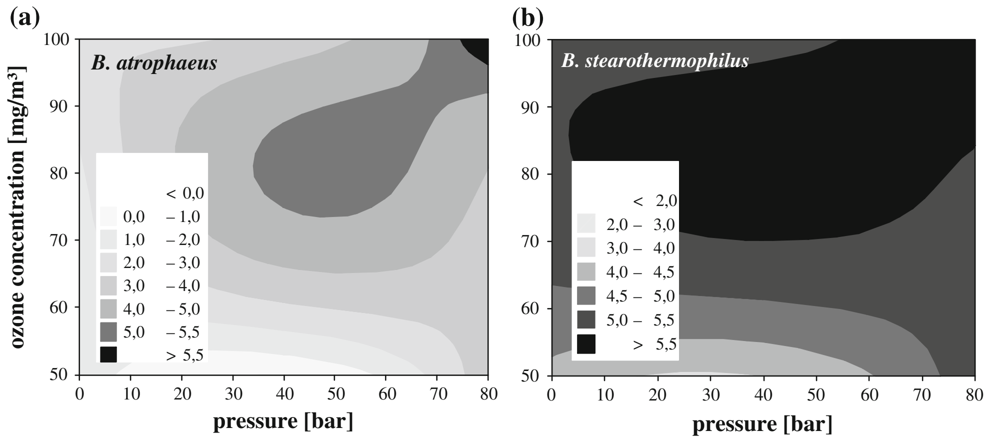

Fig. 3 Contour diagram for inactivation [log(N_0/N)] of **a** *B. atrophaeus* and **b** *B. stearothermophilus* depending on ozone concentration and pressure at 35°C and 220 min

100 mg O_3/m^3 leads to a reduction of 5.5 log cycles, and more if the pressure exceeds 80 bar (treatment time 220 min at 35°C). The optimum working condition for inactivation of both spore species results from the overlap of the black area of both contour diagrams, i.e., 80 bar and 100 mg/m^3.

It has been shown that ozone is effective as a sterilizing gas without CO_2 but is more efficient in a mixture with sub- and subcritical CO_2. From the literature it is well known that glycolipids and glycoproteins, which are essential cell compounds, are oxidized by ozone [38]. Also, Giese and Christensen [9] showed that pyrimidines and purines of nucleic acid are attacked. Enzyme activity is likewise disturbed by the reaction of ozone [20]. Therefore, is not surprising that pure ozone acts as a sterilizing agent. However, as can be seen from Fig. 3, there is also an interaction effect between CO_2 and ozone. This effect could be attributed to the influence of low pH from the formation of carbonic acid, yet the influence of pH is controversial in literature [15, 17]. Whether small acid-soluble spore proteins (SASPs) play a role, or if the compressed fluid leads to a swelling effect of the spore coats or the pressure gradient influences the permeability of the spore, has not yet been clarified. The role that CO_2 has in additive-based sterilization is much better understood for vegetative bacteria than for spores and must be further scrutinized.

Using the experimental data the constants $a_{i,j}$ in Eq. 1 can be determined and are summarized in Table 2 for *B. atrophaeus* and *B. stearothermophilus*. These factors describe the influence of the parameters, and also the interaction of these parameters on the inactivation quantitatively. As the model takes the actual physical/chemical variables into consideration and quantifies them, possible mechanisms can be discussed more easily. However, the main result is that the relationship between the parameter values and the resulting inactivation in the investigated process can be predicted mathematically, indicating that already existing HP dry-cleaning machines could be adapted for sterilization purposes. Three major (general) statements are possible: first, the inactivation factors of the compared spores differ significantly; second, without ozone, practically no measurable inactivation can be achieved; third, the interaction term between pressure and ozone is small but clearly not to be dismissed.

Table 2 Constants for the mathematical model (Eq. 1) of inactivation [log(N_0/N)] of *B. stearothermophilus* and *B. atrophaeus* for constant temperature (35°C) and treatment time (220 min)

	B. atrophaeus	*B. stearothermophilus*		Factor
a_0	−0.09631	0.00000		
a_1	−0.00068	−0.02747	x_1	Pressure
a_2	0.12012	0.02587	x_2	Ozone
a_{12}	−0.00008	0.00033	x_1x_2	Pressure/ozone
a_{11}	0.00017	0.00039	x_1^2	Pressure
a_{22}	−0.00065	0.00003	x_2^2	Ozone

Inactivation of vegetative germs and spores has been proven. However, endotoxin removal (or destruction) has to be investigated separately. If endotoxins get into the human vascular system, they control cytokine production and can cause fever or even septic shock. To evaluate the endotoxin-depleting potential of the HP technique we investigated the change in LPS concentration on contaminated meshes, including experiments that applied only ozone without CO_2 pressure. Additionally, we investigated the effect of 12-h treatment with ozone on endotoxin concentration. Results in Fig. 4 compare LPS reduction of meshes contaminated with isolated endotoxin and LPS in *E. coli*.

The results show that the concentration can be reduced, both for isolated LPS as well as LPS inoculated via intact

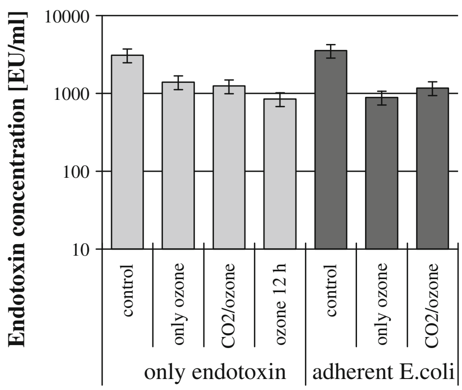

Fig. 4 Evaluation of endotoxin reduction on PP/PVDF Dynamesh® implant meshes, comparing various treatments ($n = 3$)

E. coli cells. However, reduction rates are very low, and therefore the presented HP methods are unsuitable for disintegrating, rinsing or extracting high initial concentrations of endotoxin. Successful extraction using scCO_2 as claimed by Rybka et al. [35] (they do not state whether the removal of LPS is based on dissolution or caused due to rinsing with the fluid) cannot be proven by our own results. We rather hypothesize that, in the present study, the reduction of the LPS concentration is only due to oxidative reaction of the ozone, since the experiment that was carried out for comparison with pure ozone resulted in similar reduction rates as the combined treatment. Treatment for 12 h with pure ozone does not lead to significant increase in LPS reduction.

The concentration limit for applications in the blood system has been fixed at five endotoxin units (EU) per kg body weight by *European Pharmacopeia* [34]. Therefore, we have to conclude that, at the high LPS concentrations applied in this study, insufficient reduction can be achieved, and the technique is not shown to be any better than conventional methods in this regard. LPS is removed by very few sterilization processes [7, 18]. Even ultrafiltration and ion-exchange approaches often fail and, as LPSs are heat stable, they are only inactivated at temperatures above 220°C, not suitable for biomaterial sterilization.

Conclusions

We would like to underline five main findings of this study concerning HP CO_2-assisted sterilization of medical objects and than point out its major advantages.

1. The inactivation rate depends on the type of the contaminating species, which becomes evident on comparison of Gram-positive and Gram-negative bacteria, fungi, and spores. It seems that spores of *B. atrophaeus* are among the most difficult to inactivate with scCO_2. Thus, they should be used as bioindicators for inactivation by HP CO_2 sterilization.
2. Few publications have investigated HP treatment of solid materials. We show here that also objects with inhomogeneous structures such as textiles can be successfully sterilized at low temperature.
3. The packaging of sterilized objects has so far received little attention. This study indicates that, with the use of CO_2 fluid as inactivating agent, it is possible to treat prepacked objects. Commercially available packages with a paper membrane (which is easily penetrated by the fluid) and a clear HDPE film can be applied.
4. Furthermore, it has been shown that ozone is effective as a sterilizing gas without CO_2 but is more efficient in a mixture with sub- and scCO_2.
5. Acceptable reduction of endotoxins fixed to polymers for biomedical applications cannot be expected with realistic treatment times with either ozone or the combined approach.

The outcomes of this research show that HP CO_2 in combination with O_3 can successfully be used to sterilize implantable polymers. The optimum pressure and temperature settings found in this study are consistent with adaptation of already existing dry-cleaning devices for sterilization purposes. Since production of ozone as well as elimination after treatment takes places on-site, and CO_2 can be recycled, this innovative sterilization method is a virtually zero-emission process, unlike most conventional methods. Positive results indicate that this ecofriendly treatment is a genuine alternative to conventional processes and could lead to further applications within the fields of medicine, drug processing, and biotechnology.

Outlook

The presented results give valuable basic insights into the field of HP sterilization; however, further research, e.g., into the use of other fluids with advantageous critical data is needed. Particularly, more knowledge about biogenous contamination and materials to be treated has to be gained.

(Bio)polymers have become an artificial part of the cardiovascular system in pacemakers, heart valves, stents, and other implants, often as composites with metals [1]. In biotechnology, these materials are used for diagnostic applications, e.g., sensors and electrodes. Lab-on-a-chip techniques make use of glass, semiconductors, and precious metals in extremely delicate layers, for which not even resistance against conventional sterilization methods is known. Beside these materials, further research should include membrane material for medical and food industry [44], and objects with functionalized surfaces [19] such as protein-repelling or protein coatings.

We highly appreciate that the focus of research on HP (liquid) treatment is shifting from bacteria and spores towards nonliving, biogenic material such as viruses [8] and phages [41], and towards hazardous macromolecules [21]. Prions, similarly to (endo)toxins, are highly resistant, but since folding seems to influence the infectiousness of those macromolecules, research correlating conformational changes with pressure are of interest [40]. The efficacy of pressure-based methods remains largely unknown for biofilms; however, preliminary findings indicate that they are more resilient than suspended cells [30].

Besides, CO_2 shows great versatility, enabling integrated processes such as sterilization, impregnation, extraction, and foaming in one reaction chamber.

Acknowledgments For financial funding we would like to thank the German Ministry of Education and Research (BMBF) (Project No. 0330470) and the German Environmental Fund (DBU) (Project No. 25131).

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