Biocontrol Science, 2003, Vol. 8, No. 2, 69−76

Original

Several Factors Affecting Ozone Gas Sterilization

MIEI SAKURAI [1], RYOJI TAKAHASHI [1], SAKAE FUKUNAGA [1], SHIGEFUMI SHIOMI [1], KOJI KAZUMA [2], AND HIDEHARU SHINTANI [3]*

[1] *Ishikawajima Harima Heavy Industry Co. Ltd., 1 Shinnakaharamachi, Isogo-ku Yokohama 235-8501,*
[2] *K2 International Co. Ltd., 3-13-3 2nd Hirota Bld., Kayabacho, Nihonbashi, Chuoh-ku, Tokyo 103-0025, and*
[3] *National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan*

Received 25 June 2002/Accepted 25 November 2002

Ozone gas sterilization was confirmed using commercially available as well as homemade biological indicators (BIs) utilizing *Geobacillus stearothermophilus* (formerly it was classified as *Bacillus stearothermophilus*) ATCC 12980 or ATCC 7953. The relationship between the ozone gas concentration, relative humidity or temperature and decimal reduction value (D) of BI was studied. We also investigated how the ozone gas sterilization performance was affected by the difference in the characteristics of the carrier materials of BIs. The carrier materials used were synthesized polymers with different characteristics and stainless steels (SUSs) with different levels of surface roughness and cleanness. Effect of the difference in the ATCC number of the BI on the D values was also studied. There existed a satisfactory relationship between temperature, humidity and ozone gas concentration and D value ($r>0.9$). Results indicated that D values from BIs with carrier materials of more hydrophilic materials were greater. In addition, the D value of the BI with a carrier material made of SUSs was greater than that made of polymers. The difference in the roughness of the surface of the SUS material did not cause any significant difference in D values. On the contrary, the difference in the cleanness caused a difference in D values. The difference in the SUS components, i.e. Fe, Ni, may cause some differences in D values. Resistance of *G. stearothermophilus* ATCC 12980 was around two times greater than that of ATCC 7953 when expressed as CT (concentration X time).

Key words : Ozone gas sterilization/Decimal reduction value/Carrier material/Stainless steel/Synthesized polymers.

INTRODUCTION

Ozone gas sterilization is now often conducted at health care facilities and in the health care product industries, especially, for example, in the sterilization of isolators (Berrington and Pedler, 1998; Bocci, 1996; Gal et al., 1992; Karlson, 1989; Sugahara, 1988; Suzuki et al.,1999; Weavers and Wickramanayake, 1999). In this study, ozone gas sterilization validation was performed using commercially available biological indicators (BIs) consisting of *Geobacillus stearothermophilus* (formerly it was classified as *Bacillus stearothermophilus*) ATCC 12980 or ATCC 7953 with a population of 10^3 to 10^5 cfu (colony forming unit)/sheet (ISO 11138-1). 10^3 to 10^5cfu/sheet of BI was used for confirmation of sterility assurance following the combined method of the biological indicator and bioburden (ISO 11138-1). In this paper, several factors affecting ozone gas sterilization were studied.

The relationship between ozone gas concentration, relative humidity (RH) or temperature within the sterilizer and the decimal reduction value (D) of BI

*Corresponding author. Tel : +81-3-3700-9268, Fax : +81-3-3707-6950.

was studied. We also studied how the ozone gas sterilization performance was affected by the difference of the chemical characteristics of the carrier materials of BIs. This study is definitely necessary because there is no way to be informed in advance the location where the bioburden (spoiled microorganisms) exists. If the resistance of the bioburden differs depending on the difference of characteristics of the carrier material, knowledge of this phenomenon will greatly benefit the sterilization validation of health care products.

We investigated whether the D value of BI might depend on the hydrophobicity or the hydrophilicity of the carrier material of the synthesized polymers and stainless steel (SUS) used. For that purpose, the membrane thickness, pore size and diameter and so on (physical factors) of the carrier materials used were almost identical (membrane thickness 125 μ m, pore size 0.45 μ m, and diameter of 25mm) with difference only in the chemical characteristics, hydrophobicity or hydrophilicity, of carrier materials. The D value difference was studied by using different carrier materials.

As a biological factor, the difference of the ATCC number of *G. stearothermophilus*, i.e, ATCC 12980 and 7953, was studied regarding how it might affect the D value.

MATERIALS AND METHOD

Ozone gas generator and monitor

An ozone gas generator used was an ozonizer M6[R] (Ishikawajima Harima Heavy Industry Co., Ltd.). Ozone gas concentration within the gas chamber was monitored by using an ozone monitor (EG-2001, Ebara Jitsugyo Co., Ltd.). Confirmation of the concentration of ozone gas generated was conducted by using EG-500H (Ebara Jitsugyo Co., Ltd.). Constant temperature and constant humidity was coordinated with a bath (SE24BL-A with a capacity of 288L, Kato Co., Ltd.).

Commercially available BI

Commercially available BI (Carrier material: polystylene, Steris Co., Ltd.) inoculated *Geobacillus stearothermophilus* ATCC 12980 or ATCC 7953 possesses an initial population of 10^3 to 10^5cfu/sheet with a primary package with the front surface from Tyvek[R] and the back surface from polyetheylene.

Cultivation conditions

The culture medium used was SCDB (Soybean Casein Digest Broth, Difco Co., Ltd., lot no. 138573XE). It was cultivated at 55℃ for 7 days.

D value determination

D value determination was conducted with a fraction negative method (Stumbo-Murphy-Cochran Procedure, ISO 11138-1). According to the requirements of ISO 11138-1, 20 BIs were used for 1 test. More than 2 test runs were conducted to confirm the reproducibility of the results.

Homemade BIs

In this experiment, several carrier materials were used as follows: Hydrophobic polyvinylidene difluoride (PVDF) from Millipore Co., Ltd. was treated with autoclave sterilization prior to the sterilization validation experiment (membrane thickness 125 μ m, pore size 0.45 μ m and diameter of 25mm). Hydrophilic PVDF from Millipore Co., Ltd. was treated with autoclave sterilization prior to the sterilization validation experiment (membrane thickness 125 μ m, pore size 0.45 μ m and diameter of 25mm), hydrophobic polycarbonate (PC) from Millipore Co., Ltd. was treated with autoclave sterilization prior to the sterilization validation experiment. Hydrophobic polytetrafluoroethylene (PTFE) from Millipore Co., Ltd. was treated with autoclave sterilization prior to the sterilization validation experiment. Hydrophobic polyethersulfone (PES) from Nihon Pall Co., Ltd. was treated with autoclave sterilization prior to the sterilization validation experiment (membrane thickness 97-155 μ m, pore size 0.45 μ m and diameter of 25mm). Hydrophobic acrylonitrile butadine copolymer (AN) from Nihon Pall Co., Ltd. was treated with ozone gas sterilization prior to the sterilization validation experiment at the condition of 15,000ppm, 90%RH, 35℃ and 10min (membrane thickness 127-259 μ m, pore size 0.45 μ m and diameter of 25mm). Polystylene (PS) from Steris Co., Ltd. was treated with ozone gas sterilization prior to the sterilization validation experiment at the condition of 15,000 ppm, 90%RH, 35℃ and 10min. SUSs from Amsco Co., Ltd., from Raven Co., Ltd., and from Epecus Co., Ltd. were treated with autoclave sterilization after ultrasonic cleaning. SUS from Nihon Yakin Co., Ltd. was treated with several procedures tolerable to the materials prior to the use for sterilization validation.

RESULTS

Effect of treatment conditions on D values

The relationship between the D value and an ozone gas concentration was studied. The experiment was carried out at 25℃ at RH of 80% using BIs from Steris Co., Ltd. The results are shown in Fig. 1 (a).

The relationship between the D value and RH was studied. The experiment was carried out at 25℃ at an ozone gas concentration of 15,000 ppm using BIs from Steris Co., Ltd. The results are shown in Fig. 1 (b).

The relationship between the D value and the sterilization temperature was studied. The experiment was carried out at an ozone gas concentration of 15,000 ppm and RH of 90% using BIs from Steris Co., Ltd. The results are shown in Table 1 and Fig. 1 (c).

The relationship between the D value and the different carrier materials used for homemade BIs was studied. The results are shown in the case when carrier materials used for homemade BIs were PVDF and PTFE. The results are shown in Table 2.

The D value difference according to the difference in carrier materials including synthesized polymers

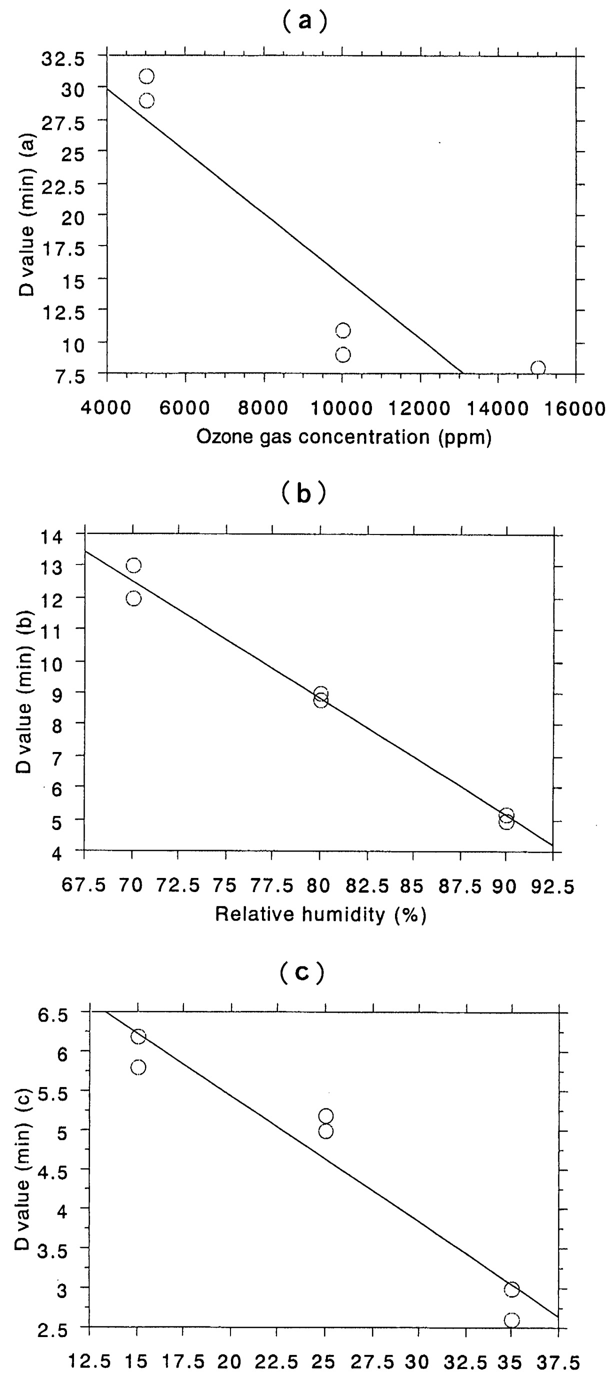

FIG. 1. Relationship between the D value and ozone gas concentration (a), between the D value and relative humidity (b) and between the D value and sterilization temperature (c). *r* for (a) to (c) was 0.881, 0.991 and 0.955.

TABLE 1. Relationship between the D value and sterilization temperature.

Temperature (℃)	Initial population (cfu)	D value (min)
15	2.9×10^5	6.2
15	1.8×10^5	5.9
15	2.7×10^5	5.9
25	1.8×10^5	5.3
25	1.8×10^5	4.6
25	2.7×10^5	5.0
35	2.9×10^5	3.0
35	1.8×10^5	2.4
35	1.8×10^5	2.3
35	2.7×10^5	3.0

The experiment was carried out at an ozone gas concentration of 15,000ppm and relative humidity of 90% using BIs from Steris Co., Ltd. Coefficient of the relationship (*r*) obtained was 0.99.

TABLE 2. Relationship between the D value and the difference in carrier materials used for homemade BIs.

Carrier materials	Initial population (cfu)	D value (min)
PVDF	1.7×10^5	4.8
PVDF	2.4×10^5	5.1
PTFE	1.7×10^5	6.0
PTFE	2.4×10^5	6.0

The experiment was carried out at 25℃ at an ozone gas concentration of 15,000ppm and RH of 80% using homemade BIs from *G. stearothermophilus* ATCC 12980 with an initial population of 10^5cfu/carrier. Carrier materials were PVDF and PTFE and the front surface of the primary package was Tyvek[R] (45×75mm) and the back surface of the primary package was polyethylene.

TABLE 3. Difference in the D value depending on the difference in carrier materials.

Manufacturer	Carrier material	Ave. D value (min)
Epecus	SUS	8.9
Raven	SUS	8.5
Amsco	SUS	5.1
Millipore	Hydrophilic PVDF	2.3
Steris	PS	<2.3

BI was homemade with an initial population of 2.3×10^3cfu/carrier. The primary package was Tyvek[R] (45×75mm) and the back surface of the primary package was polyethylene.

Sterilization conditions: 35℃ at an ozone gas concentration of 6,000ppm and RH of 90%.

D value data is the average of three figures.

and SUS was studied. The results are shown in Table 3.

Effect of polymer characteristics on the D values

The effect of hydrophilicity and/or hydrophobicity of the synthesized polymers used for carrier materials on D values was studied. In this case, membrane pore size and thickness are not always identical among polymers used for the experiment. The results are shown in Table 4.

The experimental conditions are described in the Table footnotes.

In order to confirm the effect of different levels of surface roughness and/or cleanness of the carrier material on D values, the experiment was conducted with SUSs from Epecus Co., Ltd. and Nihon Yakin Co., Ltd. with different surface roughness at the μm order.

SUSs from Epecus Co., Ltd. and Nihon Yakin Co., Ltd. were treated with autoclave sterilization after ultrasonic cleaning. In addition, in case of SUS from Nihon Yakin Co., Ltd., in advance of these treatments, the material surface was treated with acid, buff polishing, buff polishing combined with an electric polishing and degreasing treatment. The results are shown in Table 5.

The experimental conditions are described in the Table notes.

In order to confirm the definite effect of hydrophilicity or hydrophobicity of the polymer materials on the difference of D value, the polymer materials with almost identical membrane pore size (0.45μm), thickness (125μm) and diameter (25mm) were used and the difference in the D values among them was studied. The results are shown in Table 6.

The experimental conditions are described in the Table notes.

The effect of different polishing procedures on D values was studied using SUSs from Nihon Yakin Co., Ltd. (10×10×2mm). SUS from Nihon Yakin was treated with autoclave sterilization after ultrasonic cleaning. SUSs used were SUS 304 and SUS 316L, which are generally used for the interior of several types of medical equipment. The chemical components of SUS 304 and SUS 316L are presented in Table 7. The total amounts of Fe and Ni in SUS 304 and SUS 316L are almost identical and make up almost 80%.

Polishing procedures were as follows: without polishing, polishing with buff polishing (#400), buff polishing (#400) combined with the electric polishing.

TABLE 4. Effect of the hydrophilicity and/or hydrophobicity of the polymers with different membrane pore sizes and thicknesses on D values.

RH	Manufacturer	Carrier material	Ave. D value (min)
80%	Millipore	Hydrophilic PVDF	10.9
	Millipore	Hydrophobic PVDF	8.6
	Millipore	PC	5.6
	Steris	PS	5.7
90%	Millipore	Hydrophilic PVDF	>3.4
	Millipore	Hydrophobic PVDF	3.3
	Millipore	PC	<2.3
	Steris	PS	2.3

Bis were homemade with an initial population of 1.7 to 2.3×10^{3}cfu/carrier. The primary package was Tyvek^R (45×75 mm) and the back surface of the primary package was polyethylene.

Sterilization conditions: 35℃ at an ozone gas concentration of 3,000 ppm.

D value data is the average of three figures.

TABLE 5. Effect of the difference in surface roughness and/or cleanness of the carrier material on D values using different SUS materials.

Manufacturer	Carrier material	Ave. D value (min)
Epecus	SUS	8.8
Nihon Yakin	SUS	8.8

BIs were homemade with an initial population of 1.7 to 1.9×10^{3}cfu/carrier. The primary package was Tyvek^R (45×75 mm) and the back surface of the primary package was polyethylene.

Sterilization conditions: 35℃ at an ozone gas concentration of 6,000 ppm and RH of 90%.

D value data is the average of three figures.

TABLE 6. Effect of hydrophilicity or hydrophobicity of the polymer materials with almost identical membrane pore sizes, thicknesses and diameters on D values.

Manufacturer	Carrier material	Ave. D value (min)
Millipore	Hydrophobic PVDF	15.0
Nihon Pall	Hydrophobic PES	total death
Nihon Pall	AN	16.5

BI was homemade with an initial population of 1.7×10^3 cfu/carrier. The primary package was Tyvek[R] (45×75mm) and the back surface of the primary package was polyethylene.

Sterilization conditions: 35℃ at an ozone gas concentration of 3,000 ppm and RH of 80%.

D value data is the average of three figures.

TABLE 7. Chemical components of SUS 304 and SUS 316L.

SUS No.	Components (%)					
	Mn	Ni	Cr	Mo	Cu	Fe
SUS 304	0.96	8.05	18.09	0.19	0.19	71.76
SUS 316L	1.20	12.05	17.26	2.05	—[a]	66.70

The total amounts of Ni and Fe in SUS 304 and SUS 316L were almost identical and make up 80%.

[a]Not contained.

The results are shown in Table 8.

The experimental conditions are described in the Table footnotes.

Effect of strain type of *G. stearothermophilus* on D values

In order to confirm the effect of different ATCC numbers of *G. stearothermophilus* from Steris Co., Ltd. on D values, two sorts of commercially available BIs were tested. The results are shown in Table 9.

DISCUSSION

As shown in Fig. 1 and Table 1, the greater the ozone gas concentration, the RH or temperature is, the smaller the D value is. The coefficient of the relationship (r) between D vale and ozone gas concentration, the RH and temperature was 0.881, 0.991 and 0.955 (Fig. 1 (a), (b), (c)), respectively, which were satisfactory. These results indicate the confirmation of the sterilization with less time can be attained with greater gas concentration, higher humidity and higher temperature. However, too high temperature may decompose ozone gas.

As far as the experiment conducted herein, the satisfactory relationship between temperature and D value was attained as shown in Table 1 and Fig. 1 (c) (r=0.955, n=3). In general with some limitation, the higher the temperature is, the greater the degree of sterilization is as indicated in Table 1 and Fig. 1 (c). This phenomenon coincided with the Arrhenius equation. As far as this experiment goes, the possibility of decomposition of ozone gas by higher temperatures up to 35℃ can be denied due to the satisfactory relationship between the temperature and D value (Fig. 1 (c), r=0.955). At 35℃, the D value attained was around 3 min (Table 1, and Fig. 1 (c), n=3), which is the smallest D value among those generated with 15 ℃ (D value= 6min), 25℃ (D value=4.8min) and 35 ℃ (D value=2.8min).

TABLE 8. Effects of different polishing procedures on D values.

Carrier material	Polishing procedure	Ave. D value (min)
SUS 316L	Without polishing	9.4
SUS 316L	Buff polishing	8.5
SUS 316L	Buff polishing+electric polishing	8.2
SUS 304	Without polishing	8.0
SUS 304	Buff polishing	8.6
SUS 304	Buff polishing+electric polishing	7.9

BI was homemade with an initial population of 1.7×10^3 cfu/carrier. The primary package was Tyvek[R] (45×75mm) and the back surface of the primary package was polyethylene.

Sterilization conditions: 35℃ at an ozone gas concentration of 6,000 ppm and RH of 90%.

D value data is the average of seven figures.

TABLE 9. Effect of strain of *G. stearothermophilus* from Steris Co., Ltd on D values.

Strain.	Ave. D value (ppm×min)
12980	22,768
7953	12,786

G. stearothermophilus ATCC 12980 (Lot. Number 502212) from Steris Co., Ltd. with an initial population of 2.7×10^5cfu/carrier and *G. stearothermophilus* ATCC 7953 (Lot. Number F00014) from Steris Co., Ltd. with an initial population of 1.5×10^5cfu/carrier were used. Primary package was Tyvek[R] and the carrier material was polystylene.

Sterilization conditions: 35℃ at an ozone gas concentration of 5,000 ppm and RH of 90%.

D value data is the average of five figures.

More hydrophobic PVDF indicated smaller D values than PTFE did as shown in Table 2. In addition, there existed no significant difference in D values even though there was a difference in the initial population. This phenomenon was especially true in PTFE in Table 2. This is the reason there might not be any clumps of microorganisms on the BI surface, indicating inoculated microorganisms were scattered evenly on the surface of the BI carrier material.

As shown in Table 3, there was a D value difference between SUS and synthesized polymers. The D value of the BI with the carrier material from SUS was greater than that from synthesized polymers. In addition, there was a D value difference among SUSs as well as among the synthesized polymers used. There is no definite reason why the D value from SUS was greater than that from synthesized polymers. One major reason could be chemical components contained in SUS such as Fe, Ni, Mn, Co or any other inorganic /organic components may contribute as a catalyst for the decomposition of ozone gas during sterilization, which could result in a greater D value in SUS than in polymers. The definite reason for the D value differences among SUSs from Epecus, Raven and Amsco has not been clarified yet. This is because concrete information on components contained in these materials is not available from these companies and it is hard for the authors to discuss this matter without any useful information.

As shown in Tables 4 and 5, the more hydrophilic the polymer materials are, the greater the D value is. This phenomenon was especially true in case of PVDF in Table 4. In case of PVDF in Table 4, membrane thickness (125 μm), pore size (0.45 μm) and diameter (25mm) are identical as described in Materials and Methods. The magnitude of the membrane thickness, pore size and diameter as well as hydrophobicity and hydrophilicity of PC and PS differed from those of PVDF in Table 4.

The major difference between upper and lower in Table 4 is RH. Greater RH indicated smaller D values, which coincided with the results in Fig. 1 (b).

It is still not yet definitely indicated why greater D values were generated when the carrier materials were more hydrophilic. One possible speculation is as follows: the outer surface characteristics of *G. stearothermophilus* spore is relatively hydrophilic, therefore the spore can penetrate much deeper into the interior of hydrophilic polymer materials. This may result in more tolerance and a greater D value to ozone gas than hydrophobic polymer materials because ozone gas penetration capability is not significant. This speculation can be confirmed from the comparison of tolerance behavior between *G. stearothermophils* and *B. atrophaeus* (formerly it is called *B. subtilis*) by using hydrophilic or hydrophobic materials. This is because the most outer layer of *B. atrophaeus* spore is more hydrophobic than *G. stearothermophilus*. However, the resistance to ozone gas sterilization of *B. atrophaeus* is much weaker than that of *G. stearothermophilus*, therefore a significant difference in resistance between hydrophobic and hydrophilic materials was not attained when *B. atrophaeus* was used. It is difficult to experimentally confirm this speculation. As another speculation, a greater D value from hydrophilic materials was thought to be due to the possibility of the hydrophilic material's ability to hydrogen-bond with the OH radical from the ozone gas sterilant. This may result in greater consumption of the ozone gas sterilant and a greater D value.

In the experiment of Table 4, physical factors such as pore size, membrane thickness and so on were not always identical among PVDF, PC and PS, but the result of greater D values from more hydrophilic polymers than hydrophobic polymers was unchanged. This phenomenon on polymer characteristics was always true as shown in Tables 3 and 4.

No significant difference in D value was observed even though there existed statistically significant differences in surface roughness at the μm level (Table 5). This may be due to two reasons as follows. One reason is that spore size is at the μm level, so the D value may not be significantly affected with the degree of difference of surface roughness at that level. Another reason is that there may not exist any significant difference in the surface cleanness between SUSs from Epecus and Nihon Yakin. However, the concrete information on cleanness of SUS from Epecus is not available, so this is only speculation. In order to confirm this speculation on cleanness, the authors thereafter conducted the experiment presented in Table 8.

There does not exist any significant difference in the D value between hydrophobic PVDF and hydrophobic AN with an identical membrane thickness, pore size and diameter (Table 6). D value results from sterilized PES was beyond our speculation even though PVDF, PES and AN have an almost identical hydrophobicity and physical parameters. We have no definite idea why the resistance of PES to ozone gas sterilization was much weaker than PVDF and AN. The speculated reason is that PES is sensitive to autoclaving as well as to ozone gas sterilization and some chemicals inhibiting bacterial growth might be produced after these sterilization procedures. PES is fabricated from bisphenol A and 4,4'-dichloro diphenyl sulfone. PC is fabricated from bisphenol A and phosgene. The inhibition chemicals generated from the sterilized PES were speculated to be bisphenol A and /or derivatives of 4,4'-dichloro diphenyl sulfone. It is now under concern. Bisphenol A may not be a major inhibitor because PC did not indicate an identical phenomenon to PES. Derivatives of 4,4'-dichloro diphenyl sulfone may be a major contributor for inhibition. This speculation must be clarified in the further study. In addition, further research will be necessary to confirm what component/components (stabilizer, antioxidant, stimulator, monomer, oligomers, etc) in sterilized PES may cause a growth inhibition of the inoculated colony. In general, PES is thought to tolerate autoclave sterilization contrary to our results.

We confirmed the decrease in colony counts after inoculating *G. stearothermophilus* ATCC 12980 onto the PES sterilized with autoclaving or ozone gas treatment. As a result, in the case of autoclave sterilization a 30% decrease and in the case of ozone gas sterilization a 90% decrease in the colony counts inoculated was observed. This indicated sterilized PES might be an inappropriate material for medical use. Ozone gas sterilization is an appropriate sterilization procedure with exception for application to natural rubber, low density polyethylene and PES. When sterilized PES was immersed into a broth culture medium such as soybean casein digest broth (SCDB), no growth was observed in our experiment so far, which indicated inhibition materials from sterilized PES may dissolve into liquid culture medium and prohibit bacteria growth. This phenomenon is true both for *G. stearothermophilus* and non-spore forming bacteria such as *Micrococcus luteus*.

PES is often used for artificial dialyzers, hollow fibers, the membrane material for sterility tests and so on. However, the toxicity problem of the sterilized PES must be solved soon in the near future. Otherwise, the patients exposed to sterilized dialyzers from PES may have a serious risk. The sterility tests using sterilized PES membrane may result in false negative data and other undesirable problems may occur from sterilized PES.

We have no definite explanation why BIs on more hydrophilic carrier material gave a greater D value than that on hydrophobic polymers. In addition, a definite explanation for greater D values from SUS than that from synthesized polymers is not presented herein. The former phenomenon may be explainable from the hydrophilicity of the outer surface of *G. stearothermophilus* spore which may allow deeper penetration into the interior of more hydrophilic polymers, which may result in more resistance to the sterilant. In addition, the hydrogen-bond between the hydrophilic material and OH radical from ozone gas may also contribute to the generation of a greater D value. The latter phenomenon involving SUSs might be explained from the quantitative difference of mostly mineral components contained in the SUS materials which destroy ozone gas. The experimental results supporting these phenomena were generally true even though physical factors such as membrane thickness, pore size and diameter, were not always identical. Therefore, the contribution of chemical factors such as hydrophilicity, hydrophobicity affecting the difference in D values is greater than that of the physical factors of membrane thickness, pore size and diameter. Definite reasons to explain the difference in the resistance of *G. stearothermophilus* on hydrophilic and hydrophobic polymers, that on SUS and polymers and that on various SUSs must be further studied.

As shown in Tables 7 and 8, no significant difference in D values between SUS 316L and SUS 304 after buff polishing and buff polishing combined with an electric polishing were observed. D values for SUS 316L and SUS 304 after each polishing procedure were 8.5, 8.2 and 8.6, 7.9 (min, $n=7$, Table 8), respectively. The lack of significant difference in D values may be due to the lack of significant differences in the mineral components of the SUSs. This speculation is from the result that the total amount of Fe and Ni in SUS 304 and 316L is almost identical and make up 80% (Table 7).

In addition, in Table 8 there is a statistically significant difference in surface roughness between surfaces receiving buff polishing or buff polishing combined with electric polishing ($p<0.05$). The order of surface cleanness according to procedures is buff polishing combined with the electric polishing > buff polishing > without polishing. The cleaner the material surface is, the smaller the D value is. This is because there is smaller residue from organic materials to destroy ozone gas. This phenomenon is especially true in SUS 316L in Table 8.

The difference in the surface roughness of the SUS material did not cause any significant difference in D values. This is especially true for SUS 304 in Table 8 as well as in the results in Table 5. However, the difference of cleanness depending on difference in polishing procedures caused a difference in D value. The difference in SUS components may cause some difference in D values. To confirm this, further research on difference in resistance will be required by using SUSs with significantly different concentrations of mineral components. However, this kind of research may not be useful and practical, even though it is interesting academically.

Contrary to PES which was sensitive to several sterilization procedures, SUS is an appropriate material for medical use in the interior of the isolator or the interior of several types of medical equipment as there exists no significant deterioration/corrosion after a long period of use. SUS is quite sensitive to NaClO treatment rather than ozone treatment for corrosion. In a short period, SUS is deteriorated and darkened with the disinfection by NaClO treatment.

As shown in Table 9, there is a significant difference in D values between ATCC 12980 and that of 7953. This result indicated ATCC 12980 should be utilized as BI for ozone gas sterilization because the microorganism selected for BI should be the most resistant microorganism for the sterilization procedure according to the definition of BI in ISO 11138-1. In Table 9, resistance is expressed in CT (concentration X min) and there is a difference of almost 2 times between ATCC 12980 and ATCC 7953. When this is expressed as D value (min), a difference of 3 times was attained between ATCC 12980 and ATCC 7953.

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