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Abstract

Specific treatments combining high temperatures of up to 150°C and moderate pressure of up to 0.6 MPa have been applied to *Bacillus subtilis* 168 spores conditioned at different a_w levels (between 0.10 and 0.70) corresponding to different residual water contents within the spore core. The spores were treated as a dry powder in a pressurized nitrogen environment or in water/glycerol solutions.

These thermodynamic conditions were intended to prevent any water evaporation from the spore core during time/temperature treatments.

Our results clearly show that retaining liquid water in the core by applying pressure during the treatment resulted in greater spore destruction (between 2.4 and 4.9 log at 150°C, 120 s and a_w 0.5 in powder) than the destruction observed after the treatment at atmospheric pressure (0.7 log), during which the water rapidly evaporated because its boiling point was reached.

Moreover, we found that the water activity level of the spore had a significant impact on spore destruction: the higher the a_w level, the greater the spore inactivation.

We obtained similar results from spores heat-treated in powder and in water/glycerol solution at the same a_w, confirming the strong influence of this parameter. We hypothesized that the increased spore inactivation was related to the well-known thermal sensitivity of vital organic molecules such as proteins, enzymes, and ribosomes in the presence of water.

Keywords

Bacillus spores – dry food – food powder - thermal sterilization – pressure – water activity

1. Introduction

Low water activity foods (a_w <0.6), such as food powders, seeds, dried fruits, cakes, flakes, spices and aromatic herbs, represent a large amount of food products consumed directly or used as intermediate food products (Cuq et al., 2011). These products contain vegetative and sporulated bacteria that cannot grow due to the low water activity level. Safety problems can arise after rehydration before or during ingestion. To prevent such incidents, these products must be decontaminated, but heat decontamination is more difficult for dry products than it is for liquid products. Indeed, dried microorganisms are more heat-resistant (Grasso et al., 2014; Laroche and Gervais, 2003) and heating without water could also rapidly cause burning damage to the product. Among dried microorganisms, sporulated bacteria are particularly resistant (Tiburski et al., 2014) and spores of *Bacillus* species can survive various drying and heat treatments used in the food industry (Andersson et al., 1995). These spores have also been seen to adhere to stainless steel and to resist cleaning in place (CIP) procedures in and around food factories (Tauveron et al., 2006). These characteristics have obvious implications, from equipment bacteria could contaminate food and therefore cause high numbers of spores to be found in food products. Dried foods such as spices, milk powders and cereal products are often quite heavily contaminated with spores, and when water becomes available during food preparation these spores may germinate, leading to spoilage or food poisoning (Logan, 2012). However, destroying bacterial spores is rather difficult, and most of the techniques commonly used to treat dry foods result in very low spore inactivation (Silva et al., 2013).

The most frequently used decontamination techniques for dry products are steam thermal processing, irradiation and fumigation. However, steam adds undesirable moisture to dry foods and causes color changes. Fumigation has been banned in most countries due to its toxicity, and irradiation is strongly rejected by consumers. In addition, these decontamination

techniques cause loss of quality and sometimes low inactivation rates (Ghisleni et al., 2016). Therefore, there is a need to develop new technologies to produce high quality and safe dry food ingredients such as herbs and spices.

Though spices are non-perishable ingredients, once they are put in water-rich food products, their natural or contaminant flora can quickly develop and multiply, consequently putting the health of consumers at risk. This is an especially important issue for spices added to ready-to-eat foods that are not subjected to further heat treatments (Van Doren et al., 2013).

Moreover the existence of pathogenic spores like *B. cereus* strains, that may cause foodborne infections (Glasset et al., 2016) or like *Clostridium botulinum* strains that may sometimes cause infant infection through dairy products (Doyle et al., 2015), is considered as a major risk for the food industry.

It is thus important to decontaminate food powders efficiently, especially if these powders are to be incorporated into a more complex preparation with a higher water content. The decontamination of dried powders is difficult, and the difficulty correlates with the increasing resistance of spores and vegetative forms to low water activity levels (Fine and Gervais, 2005b; Laroche et al., 2005; Laroche and Gervais, 2003; Tiburski et al., 2014).
Many authors have already shown that the low water activity or low water content in spores could explain their high heat resistance and that water diffusion from or out of the spore core is slowed (Knudsen et al., 2016; Loison et al., 2013; Nguyen Thi Minh et al., 2010b). In a previous work, *Bacillus subtilis* spores have been reported to have a low water permeability and suggested that the coat structure may be necessary for maintaining this low a_w (Knudsen et al., 2016). In another manuscript, using a molecular rotor, the high viscosity of the internal membrane of this spore was demonstrated and correlated this property with its barrier properties (Loison et al., 2013).

In previous studies on spore thermal inactivation, *Bacillus subtilis* spores were shown to have D-values which fell in the same range of pathogen spores like *B. cereus* and *B. anthracis* (Montville et al., 2005). Nevertheless, large interstrain variations in spore heat resistance has been regularly reported. For example, the presence of a specific operon (SPOVA2mob not present in strain 168 used in this work) leads to increase the D value more than 100 times (Krawczyk et al., 2017).

B. subtilis is also used as a surrogate for some pathogenic spores (Hu and Gurtler, 2017). Moreover *B. subtilis* itself can induce food spoilage incidents particularly on bread (Thompson et al., 1993).

Recently Tros et al. (2017) have shown that a large fraction of "slow" intracellular water linked to proteins exists in the *Bacillus subtilis* spores and in a previous work (Tiburski et al., 2014), the role of water in the heat resistance of dried *Bacillus subtilis* spores was also investigated using Differential Scanning Calorimetry. The spore temperature was linearly increased in pans with different pressure resistances. The results demonstrated that even in dry external conditions, a water fraction stayed embedded in the spore core and that maintenance of this core water content during heating dramatically increased spore destruction. Infrared analysis of treated spores showed that the heat destruction was highly related to protein denaturation and dipicolinic acid (DPA) release, which were maximized in wet conditions.

This work was intended to apply these results to optimizing dried spore decontamination based on preserving the initial water content of the spore core. The method used to prevent water evaporation was to pressurize a hermetic container and consequently change the water vapor equilibrium.

This study investigated the inactivation of dried *Bacillus subtilis* spores equilibrated at different water activities by a treatment using high temperature and absolute pressure levels from 0.1 to 0.6 MPa. *Bacillus subtilis* spores were already used as surrogates for pathogenic spores (Hu and Gurtler, 2017). The applied pressure prevented the water in the samples from boiling, thus allowing its initial water content to be maintained. The effect of time/temperature treatments under different pressure levels and for different initial water activities on final spore viability were compared.

2. Material and Methods

2.1 Bacterial strain and growth conditions

The reference strain *B. subtilis* 168 from the BGSC (Bacillus Genetic Stock Center, Department of Biochemistry, The Ohio State University, USA) was used in this study. *B. subtilis* sporulation was induced in a complex medium in a reactor at 37°C, pH 8.0, with an air flow of 4 L/min and with agitation (450 rpm) (Nguyen Thi Minh et al., 2008). Sporulation was estimated by plating on Bromocresol Purple agar (Dextrose Tryptone agar, BIOKAR Diagnostics, Beauvais, France) before and after a heat treatment at 80°C for 10 min. When more than 95% of the population in the sample resisted this heat treatment, the spore suspension was harvested (3–5 days). The spore suspension was washed with sterile distilled water four times and was then spray-dried (Mini spray dryer B-290, Buchi, France) and stored in powder form in sterile receptacles at 4°C until use. The final microbial concentration of the powder was approximately 10^{11} CFU/g.

2.2 Equilibration of spores at different water activities in gaseous and liquid media

Dried spores with water activity values of 0.10, 0.20, 0.30, 0.40, 0.50 and 0.70 were obtained by placing the spores (±500 mg) inside 1 L air-tight plastic boxes containing the water/glycerol solutions presented in Table 1 (the solution occupied 1/10 of the box volume) until equilibrium was reached (at least one week). Water activity determinations of the osmotic solutions and spore powders were performed in triplicate at 25°C using a Decagon-AQUALAB CX-2 water activity meter (Meter, WA, USA).

For liquid media, 10 mg of spray-dried spores previously equilibrated at different water activity levels were placed directly in water/glycerol solutions at concentrations predicted by the Norrish Equation to correspond to a_w levels of 0.10 to 0.70 (Norrish, 1966). The experimental a_w values were also checked with the osmometer previously described.

2.3 Treatment of dried spores in gaseous or liquid medium at high temperature and up to a pressure level of 0.6 MPa

The reactor consisted of a small hermetic stainless-steel cylinder (1 cm^3 of volume) that could be pressurized with gas. Nitrogen was used to pressurize the reactor, after which the reactor's valve was closed to maintain the applied pressure.

2.3.1 Gaseous medium

In closed systems containing water, increasing pressure prevents water vaporization. Therefore, if the pressure in the reactor is lower than the pressure of water vaporization (P_{vap}) at the considered temperature, water vaporization from the spores will occur until P_{vap} is reached. On the other hand, as soon as the pressure exceeds P_{vap}, no vaporization of the water present in the spores will occur. The final pressure inside the reactor is determined by the input controlled pressure of nitrogen and two modifying factors: i) the pressure increase resulting from increased temperature and ii) the pressure increase resulting from water evaporation from the sample. These factors have been considered to fit the evolution of the

160 water vaporization in the reactor as a function of the total pressure evolution for different
161 initial nitrogen pressures.

162 Figure 1 models the increasing pressure in the vessel for each initial nitrogen pressure level as
163 a function of the increasing temperature and the water vaporization temperature evolution
164 curve. As soon as the temperature/pressure curve crosses the temperature vaporization curve
165 (which occurs for 0.1 MPa, 0.2 MPa and 0.3 MPa at 107, 130 and 145°C, respectively) any
166 liquid water present in the vessel will evaporate from this intersection point until the end of
167 the heat treatment. For the 3 higher initial pressure levels (i.e., 0.4, 0.5 and 0.6 MPa), the 3
168 curves did not cross the water saturation curve before a temperature of 150°C was reached in
169 the experiment (see Figure 1), thus one can assume that there was no evaporation of any
170 liquid water in the vessel from an initial pressure of 0.4 MPa.

171 For most experiments, 10 mg of dried spores were placed in aluminum foil that was folded
172 and placed inside the reactor. The reactor was then pressurized between 0.2 and 0.6 MPa,
173 closed and heated in an oil bath at 150°C. When atmospheric pressure was used, the valve
174 was left open to allow water evaporation. After the treatment, the reactors were cooled in an
175 ice bath, then the valve was opened to release the pressure.

176 2.3.2 Liquid medium

177 For one set of experiments, performed at 0.6 MPa initial pressure and 150°C, the reactor
178 described previously was filled with water-glycerol suspensions at the same five a_w values
179 previously examined in gaseous medium, and 10 mg of spores were added immediately
180 before heat treatment. Under these thermodynamic conditions, there was no vaporization of
181 the different water/glycerol solutions or of pure water.

For liquid and gaseous treatment, it was verified that such a pressure level (0.6 MPa) does not alter spore viability. In fact pressure levels greater than 200 MPa are necessary to slightly inactivate spores with gas medium (Colas de la Noue et al., 2012) and 100 MPa in liquid medium (Nguyen Thi Minh et al., 2010a).

186

2.4 Spore viability

Heat-treated dried spores and spores in liquid medium were serially diluted with physiological saline solution (0.9% NaCl w/v), and 100 µl of each dilution was seeded in triplicate onto BCP agar (Dextrose Tryptone Agar, Biokar Diagnostics, Beauvais, France) plates. Colonies were counted after 24 hours of incubation at 37°C. Control samples contained untreated spores. All experiments were performed at least in triplicate. Spore inactivation was expressed using a logarithmic reduction factor $\log (N_0\text{-}N)/N_0$, with N_0 representing the number of developing spores before treatment and N representing the number of developing spores after treatment.

2.5 Statistical analysis

On each viability point (at least 3 independent measurements) standard deviation was calculated and plotted as error bars on each figure. In order to confirm the effect of pressure level, an ANOVA and Tukey's honestly significant difference test (if $p < 0.05$) were performed to determinate whether significant differences existed among treatment at different pressure. Analyses were performed using the R software, version 3.1.2.

3. Results

3.1 Effect of temperature and pressure on the viability of dried spores in gaseous medium

Initial pressures between 0.2 and 0.6 MPa were used to study the effect of pressure combined with heat in *Bacillus* spore inactivation. Spores were equilibrated at a water activity of 0.50, which is a typical a_w for dried foods such as flours, cereals and spices (Lang et al., 2017). The heat treatment (150°C, 120 s) was chosen after preliminary tests that showed these conditions to be discriminative on the ratio of spore viability to mortality.

In Figure 2, we show that heat treatment performed with no additional pressure, i.e., 150°C at atmospheric pressure, resulted in a slight destruction level of 0.3 log, which is not significantly different from the control sample maintained at 25°C and which certainly corresponds to the inactivation of pre-germinating or germinating spores.

Thus, this treatment did not inactivate any spores, which illustrates their significant thermoresistance. The results presented in Figure 2 also clearly show that when the applied pressure was increased, spore inactivation also increased. Below 0.3 MPa, the inactivation level was found to be less than 3 log, whereas above 0.3 MPa, the inactivation suddenly increased from 4 log CFU for 0.4 MPa to 5 log CFU for 0.6 MPa.

An ANOVA performed on these results confirmed that the inactivation levels corresponding to the two pressure level groups (0.2 and 0.3 MPa versus 0.4, 0.5 and 0.6 MPa) were significantly different ($p < 0.05$).

To further probe the previous results, the kinetics of spore inactivation was studied at a_w 0.5 and 150°C. The pressure used was 0.6 MPa, which corresponded to higher spore destruction in Figure 2. The inactivation of spores with time is shown in Figure 3. Spores heated at atmospheric pressure exhibited only a slight inactivation of approximately 0.5 log after 240 s, whereas spores subjected to 0.6 MPa pressure exhibited an inactivation of 5 log between 60 s and 120 s of treatment.

Data from Figures 2 and 3 show that pressure significantly affects the destruction of *B. subtilis* spores undergoing a 150°C heat treatment at an a_w of 0.5. This pressure effect exists at all pressure levels but becomes more significant with a gap of 1 to 2 log destruction for initial pressure levels greater than 0.4 MPa.

3.2 Effect of water activity on the viability of dried spores at different times for a 150°C/0.6 MPa treatment

Dried food products and food powders normally have a water activity ranging from 0.20 to 0.50, and in exceptional cases, their a_w may reach 0.70, thus it is important to investigate the effect of this variable on the proposed treatment.

3.2.1 In gaseous medium

The influence of water activity on spore inactivation is presented in Figure 4. These results clearly show that the water activity, and consequently the water content of the spores, plays a fundamental role in spore resistance. Spores equilibrated at an a_w of 0.10 exhibited an inactivation of 5 log after 560 s, whereas spores at an a_w of 0.20 exhibited an inactivation of 8 log after the same amount of time. When water activity increased, spore inactivation also increased, as shown by the decrease in logarithmic reduction time (D-values) presented in Table 2. Indeed, the D value is four times greater for an a_w value of 0.10 than for an a_w value of 0.7. Moreover, we found that the same increase in water activity level does not result in a proportional increase in spore inactivation. For example, there is only a small difference between spore inactivation at a_w 0.30 and 0.50, but further increase to an a_w of 0.70 resulted in complete inactivation after only 240 s.

252 It is important to emphasize that, with a pressure of 0.6 MPa, the treatment times are greatly
253 reduced, and so, even for water activity levels as low as 0.10 and 0.20, it is possible to reduce
254 the spore population by a factor of 10 to 5 in approximately 9 or 6 minutes, respectively.
255
256 3.2.2 In liquid medium
257 To build on the previous results, the protective effect of a_w on spore thermal resistance was
258 analyzed in a liquid aqueous medium in the same experimental chamber used for spore
259 inactivation in a gaseous medium. Addition of glycerol in the treatment liquid medium was
260 used to control the a_w of the medium and therefore may have protected spores from heat.
261 However, osmolytes sometimes also have direct toxic effects on spores and interfere with the
262 pure osmotic effect (Mazas et al., 1999). Nevertheless, spores are known for their low
263 permeability to many toxic chemicals, in particular, those that can damage the spore DNA
264 located in the central spore core (Knudsen et al., 2016). Moreover, glycerol, which was used
265 in our experiment, is a compatible solute with very few interactions with other molecules,
266 thus it is an almost ideal solute. Therefore, its action may be mainly related to the a_w decrease.

267 The results shown in Figure 5 demonstrate that the water activity of the treatment medium had
268 a major effect on spore heat inactivation. When heated in higher water activities, spores were
269 more sensitive to heat. Spores treated at a_w 0.10 or 0.20 presented a 2 log reduction after 240
270 s, whereas spores at a_w 0.30, 0.40 and 0.50 presented a 6 log inactivation after only 180 s.
271
272 Spore inactivation began at different treatment times depending on the a_w of the medium and
273 later than we observed for spores treated in dried form (see Figure 3). This result can be
274 explained by the heating kinetics inside the reactor. Indeed, previous experiments (data not
275 shown) have allowed us to verify that the temperature inside the reactor reached 99% of the
276 oil bath temperature in less than 10 s when air/nitrogen was the transmitting medium;

however, more than 100 s were needed for pure glycerol and 60 s for pure water to reach the same 99% temperature response. Figure 5 shows that inactivation at an a_w of 0.70 was noticeable between 60 s and 90 s, whereas for lower a_w levels, corresponding to lower spore water content, inactivation started only after 120 s of treatment.

Comparing these results with those obtained from dried spores in gaseous conditions (see Figure 3), it can easily be noted that for the same water activity, the final spore inactivation was quite similar.

4. Discussion

Steam treatment is in extensive use today for microbial decontamination of dry foods, but its use presents problems since it results in water condensation on the product that must be removed to avoid subsequent mold growth and spoilage. On the other hand, thermal processes such as microwaves (Kim et al., 2009), infrared (Eliasson et al., 2014) and High Temperature Short Time treatment (Fine and Gervais, 2005a) necessarily involve supplementary drying of the product. Moreover, alternative treatments available for use in aqueous media, such as high pressure and pulsed electric fields, are not efficient for the decontamination of dried foods (Espinasse et al., 2008). Therefore, new procedures for decontaminating dried foods that can destroy bacterial spores are needed.

This study investigates the inactivation of dried *Bacillus subtilis* spores equilibrated at different water activities by a treatment using high temperature and absolute pressure levels of nitrogen from 0.2 to 0.6 MPa. In this experiment, the applied pressure prevented water evaporation from the treated samples, allowing the initial water content of the spores, which is mainly situated in the spore core, to be maintained.

Inactivation varies from less than 1 log at atmospheric pressure to more than 5 log at 0.6 MPa. Between the initial pressure level of 0.3 MPa at 25°C (which generates 0.43 MPa at 150°C) and the initial pressure 0.4 MPa at 25°C (which generates 0.57 MPa at 150°C), there is a sudden increase in inactivation, in contrast to the slower increase observed at higher pressures. Based on these results, it can be concluded that when the pressure generated in the vessel exceeded the pressure corresponding to the water vaporization temperature of 150°C (0.47 MPa, see Figure 1) the spore inactivation significantly increased, as shown in Figure 2.

From this result, it can be proposed that i) maintaining water in the endospore favors bacteria inactivation and that ii) spore drying, which is initiated for pressure levels lower than 0.47 MPa, favors bacteria survival at 150°C. Moreover, the drying time and thus the final drying level decreased with increasing pressure until no further drying occurred, when the internal pressure reached the 0.47 MPa value, which corresponded to a nitrogen pressure between 0.3 and 0.4 MPa (see Figure 1).

One can propose a role of water in spore inactivation in agreement with numerous works (Coleman et al., 2007; Sochava, 1997; Subramanian et al., 2006) in which the internal endospore macromolecules, mainly composed of DNA, DPA, and proteins (the last being the most delicate), are preferentially heat-damaged when mobile and unfolded in a water medium. Moreover, a recent work (Tros et al., 2017) has observed a large fraction of “normal” intracellular water in the spore core of *B. subtilis*.

Even for very low water activity levels, noticeable spore inactivation was obtained in liquid as well as in gaseous medium. These results agree with previous work (Tiburski et al., 2014), which has shown a residual high water content in the core even for low a_w-equilibrated spores. This phenomenon was attributed to specific high barrier properties of the internal

spore membrane slowing down the thermodynamic balance. Thus, it could be assumed that some water remaining in the core caused macromolecule denaturation.

Inactivating one or more key spore proteins is believed to be important in spore heat killing (Coleman et al., 2010). When heat surpasses a threshold level, especially in aqueous media, changes in the structure of vital growth proteins become irreversible, causing spore inactivation (Grinshpun et al., 2010). It is also known that in low moisture states the temperature of protein denaturation increases (Sochava, 1997), making inactivation of these key proteins more difficult. In this context, the high spore heat resistance at low water content can be explained by the low mobility and changes in the conformations of proteins and enzymes. Indeed, most spore proteins are rotationally immobilized, which may contribute to heat resistance by preventing heat-denatured proteins from aggregating irreversibly (Sunde et al., 2009). Consequently, when the original water content of the spore is maintained, protein damage is increased during heat treatment, and such damage is certainly relevant to the differences in spore inactivation found in our experiments. This hypothesis has been directly verified through FTIR measurement in a previous work (Tiburski et al., 2014). Nevertheless, the present results were obtained on *B. subtilis* strain 168 and it is possible that at the pressure and temperature levels used the spore inactivation mechanisms could vary for other bacterial spores.

Conclusion

The model system developed in this work can be extended to treating low water content foods. This system is based on the application of temperature under pressure, which prevents any water evaporation from bacterial spores embedded in the food product. This model has the advantage of not adding moisture to the product, and it results in high spore destruction rates (approximately 5 log). The efficiency of this process is related to the high water

permeability barrier of the internal membrane of the spore, which has been shown to allow maintenance of a core water content greater than the outer membrane long after drying. Thus, this new process is particularly efficient for dried bacteria spore destruction because of their high residual core water content. Further studies must first verify this effect on other sporulated strains and especially pathogens. In a second phase, we must integrate the impact of such pressure/temperature/time treatments on the organoleptic and nutritional properties of different dried food products. Indeed, optimal treatments should conserve the functional properties of food in addition to assuring maximal bacteria spore inactivation. Following this work, this process could then be tested to treat dried food products such as spices. The normal a_w of dried food ranges between 0.30 and 0.50, and in this work, we have shown that spore inactivation higher than 5 log can be expected from a 3 minute treatment at these water activities. Therefore, if the products already possess a similar a_w, no previous equilibration will be needed; however, if the product to be processed has a very low a_w, an equilibration step would be necessary. In this case, the dried food could be stored in an atmosphere with a relative humidity of 50% for a few weeks prior to treatment. Certainly, different conditions of time, temperature and pressure adapted to each product will need to be tested to optimize this new decontamination procedure.

The next step would be to develop a scaled-up version of the system to perform tests in real food products to evaluate both microbiological decontamination and changes in quality parameters.

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Figure 1: Calculated variations of internal pressure during isochoric heating of pressurization devices (internal volume of 1.7 ml) loaded with a 10 mg spores sample initially equilibrated at a_w 0.5 and at initial pressure ranging from 0.1 MPa to 0.6 MPa, in comparison with water boiling temperature of a model saline solution initially at a_w 0.5. (NIST Chemistry Webbook has been used for water data calculation).

Figure 2: Thermal inactivation of *B. subtilis* 168 dried spores initially equilibrated at a water activity of 0.50 treated at 150°C / 120s in a gaseous medium at different initial pressure levels. (Error bars are SD, Mean comparison using Tukey's test: same letters indicate no significant difference between treatments P>0.95)

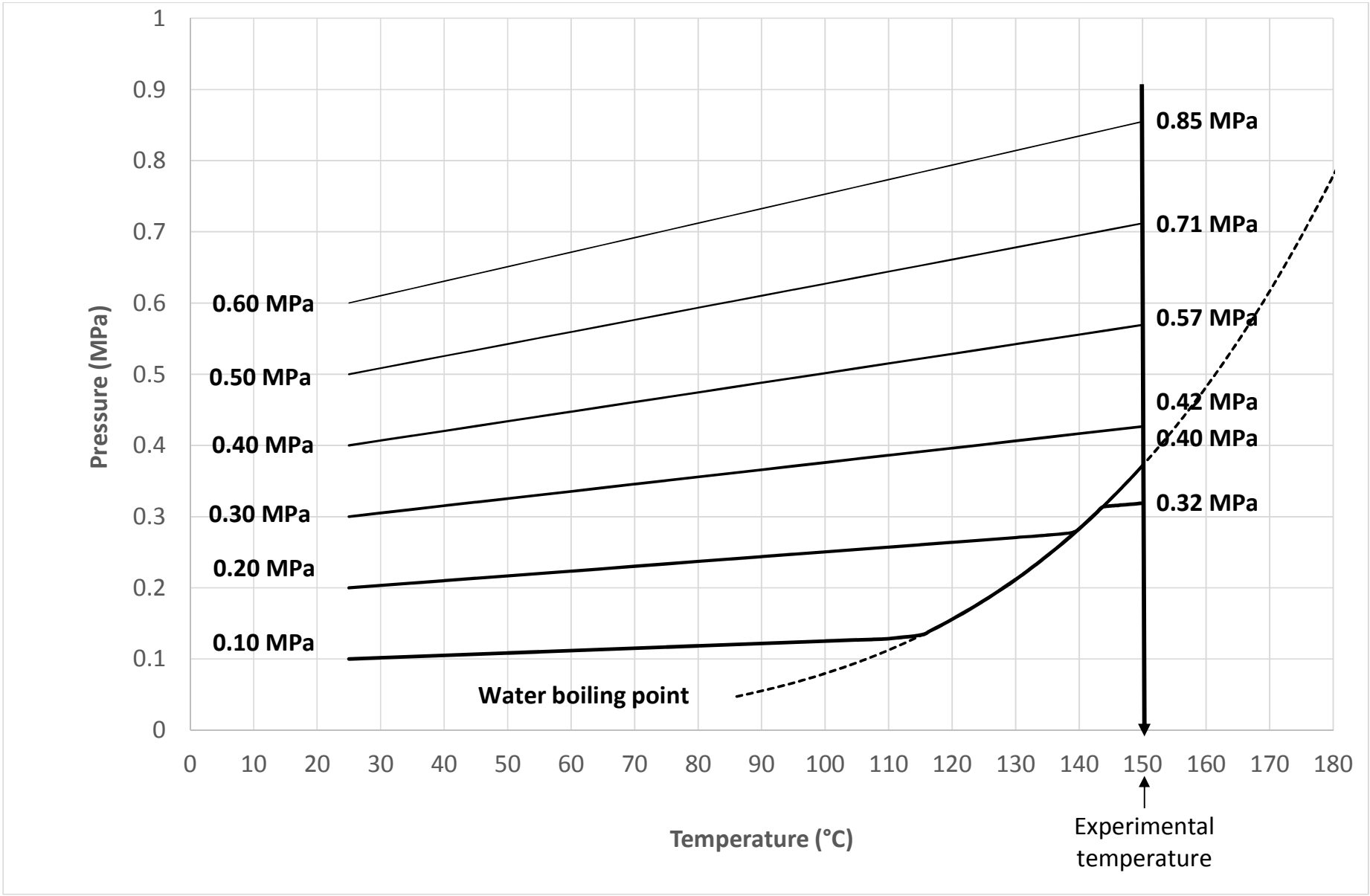
Figure 3: Kinetics of *B. subtilis* 168 dried spores' inactivation. The spores have been dried and equilibrated at a water activity of 0.50 and treated at 150°C in a gaseous medium initially at atmospheric pressure (empty triangle) or at 0.6 MPa (dot). Error bars are SD of at least 3 independent measures.

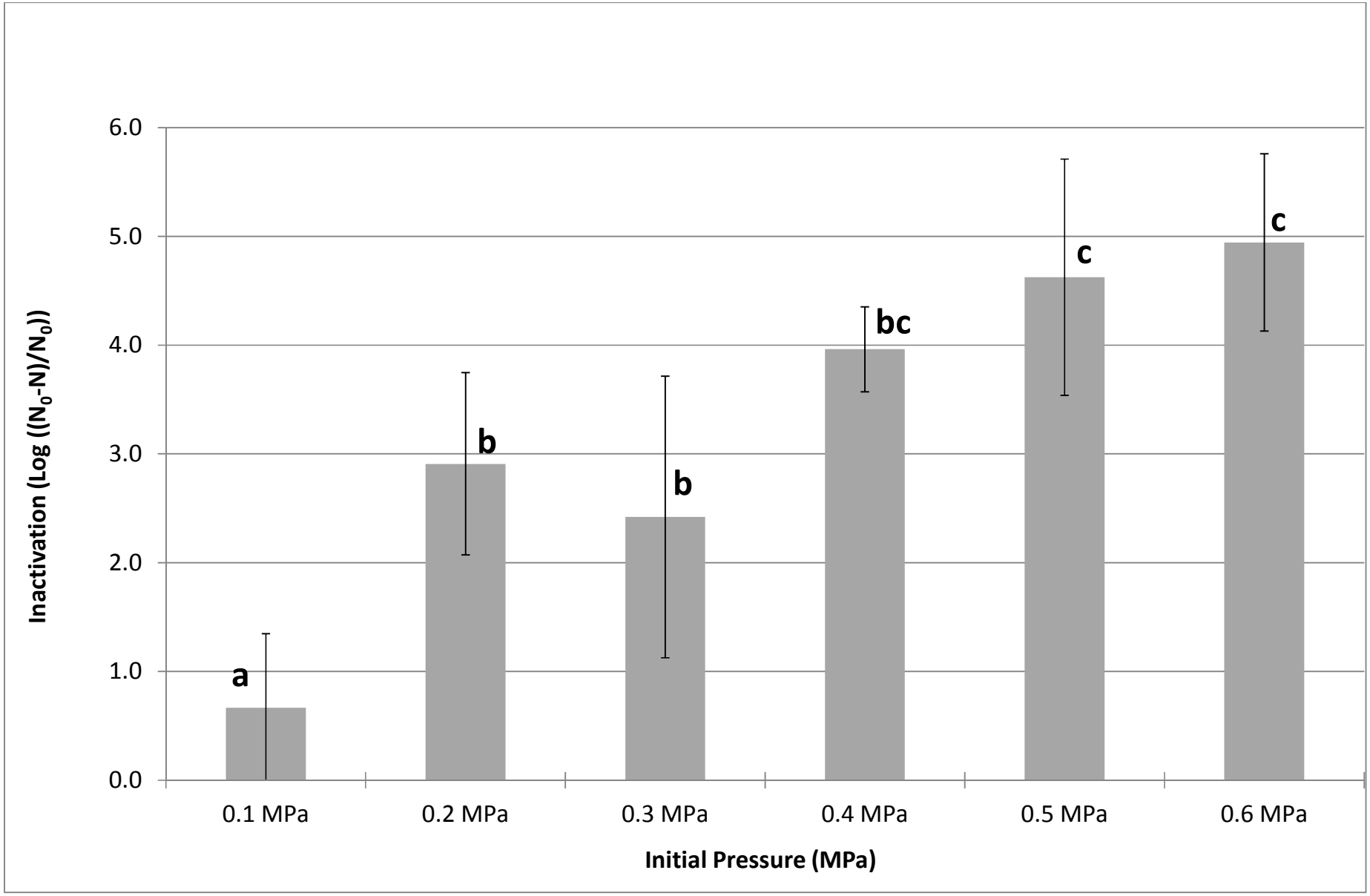
Figure 4: Inactivation of *B. subtilis* 168 spores equilibrated at different water activities (0.10, 0.20, 0.30, 0.50, and 0.70) after a treatment at 150°C and 0.6 MPa. Error bars are SD of at least 3 independent measures

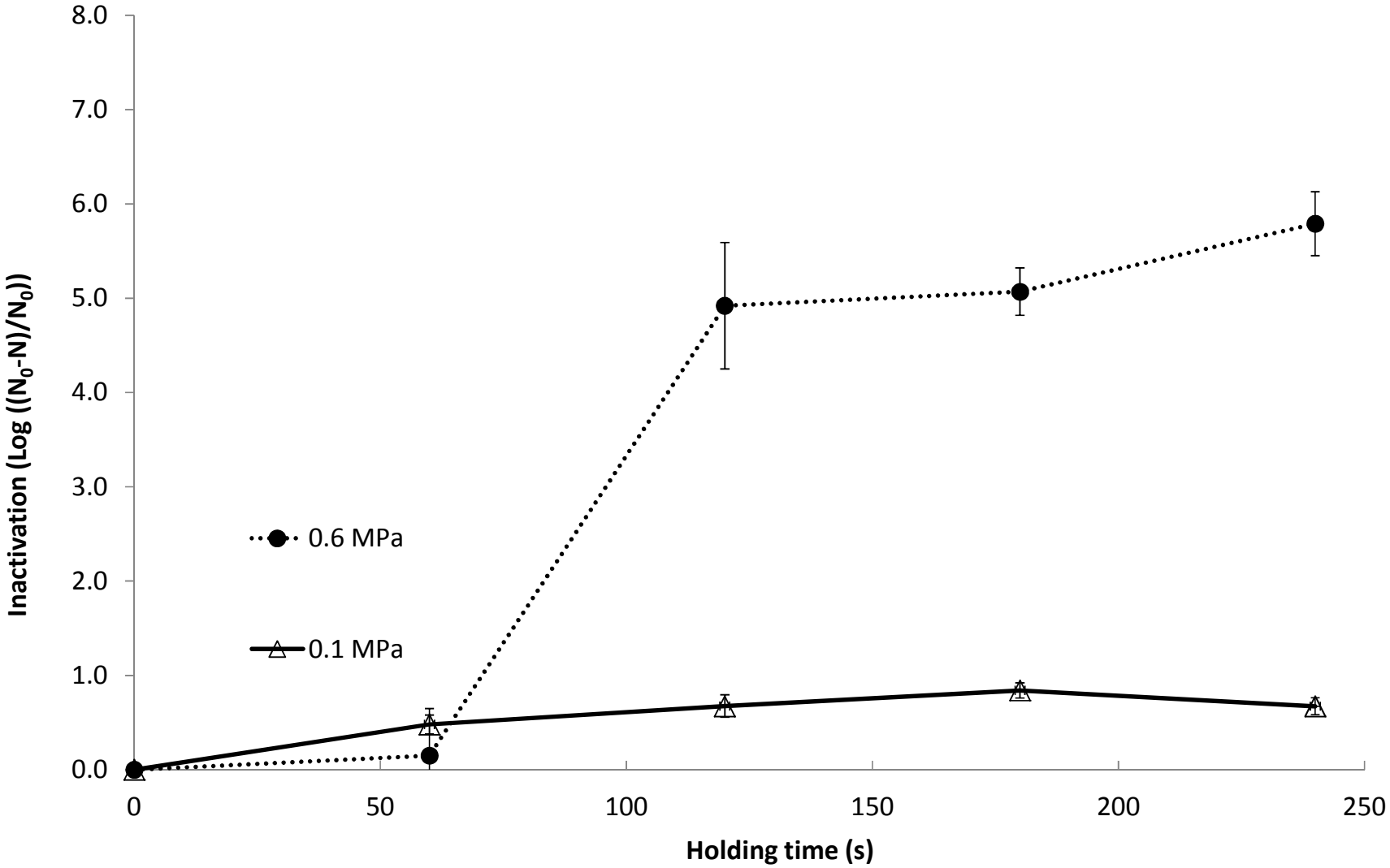
Figure 5: Inactivation of *B. subtilis* 168 spores in water/glycerol binary solutions at different water activities after a treatment at 150°C and 0.6 MPa. Error bars are SD of at least 3 independent measures.

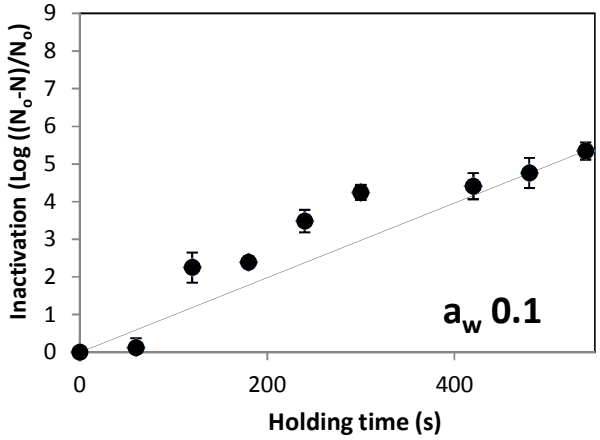
Table 1: Glycerol concentration (from Norrish equation) used to equilibrate the water activity in water/glycerol binary solutions.

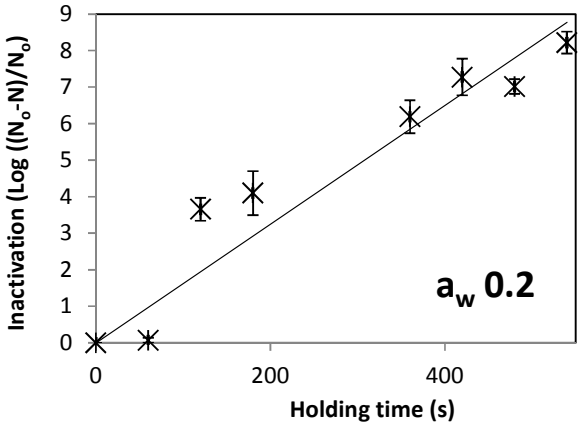
Table 2: Decimal reduction times (in minutes) for *B. subtilis 168* dried spores according to the initial water activity of gaseous environment for a treatment at 150°C and a pressure of 0.6 MPa

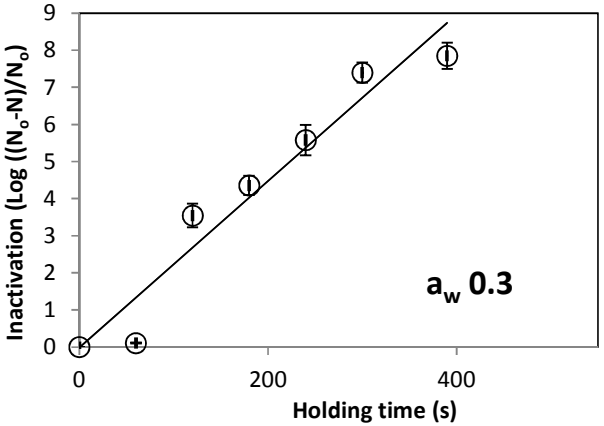

Pressure (MPa)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.60 MPa
0.50 MPa
0.40 MPa
0.30 MPa
0.20 MPa
0.10 MPa
0.85 MPa
0.71 MPa
0.57 MPa
0.42 MPa
0.40 MPa
0.32 MPa
Water boiling point
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180
Temperature (°C)
Experimental temperature

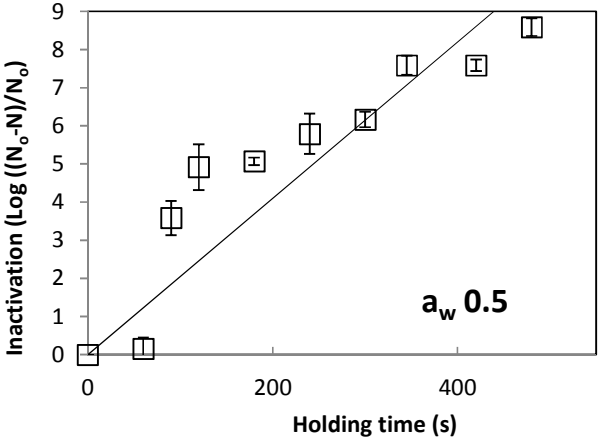

6.0
5.0
4.0
3.0
2.0
1.0
0.0
Inactivation (Log ((N0-N)/N0))
a
b
b
bc
c
c
0.1 MPa
0.2 MPa
0.3 MPa
0.4 MPa
0.5 MPa
0.6 MPa
Initial Pressure (MPa)

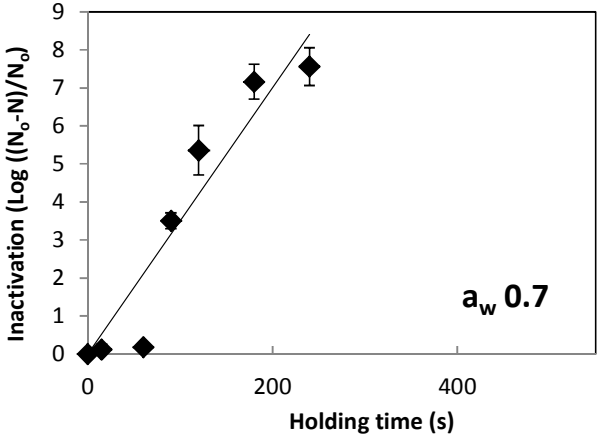

8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Inactivation (Log ((N0-N)/N0))
0.6 MPa
0.1 MPa
0
50
100
150
200
250
Holding time (s)

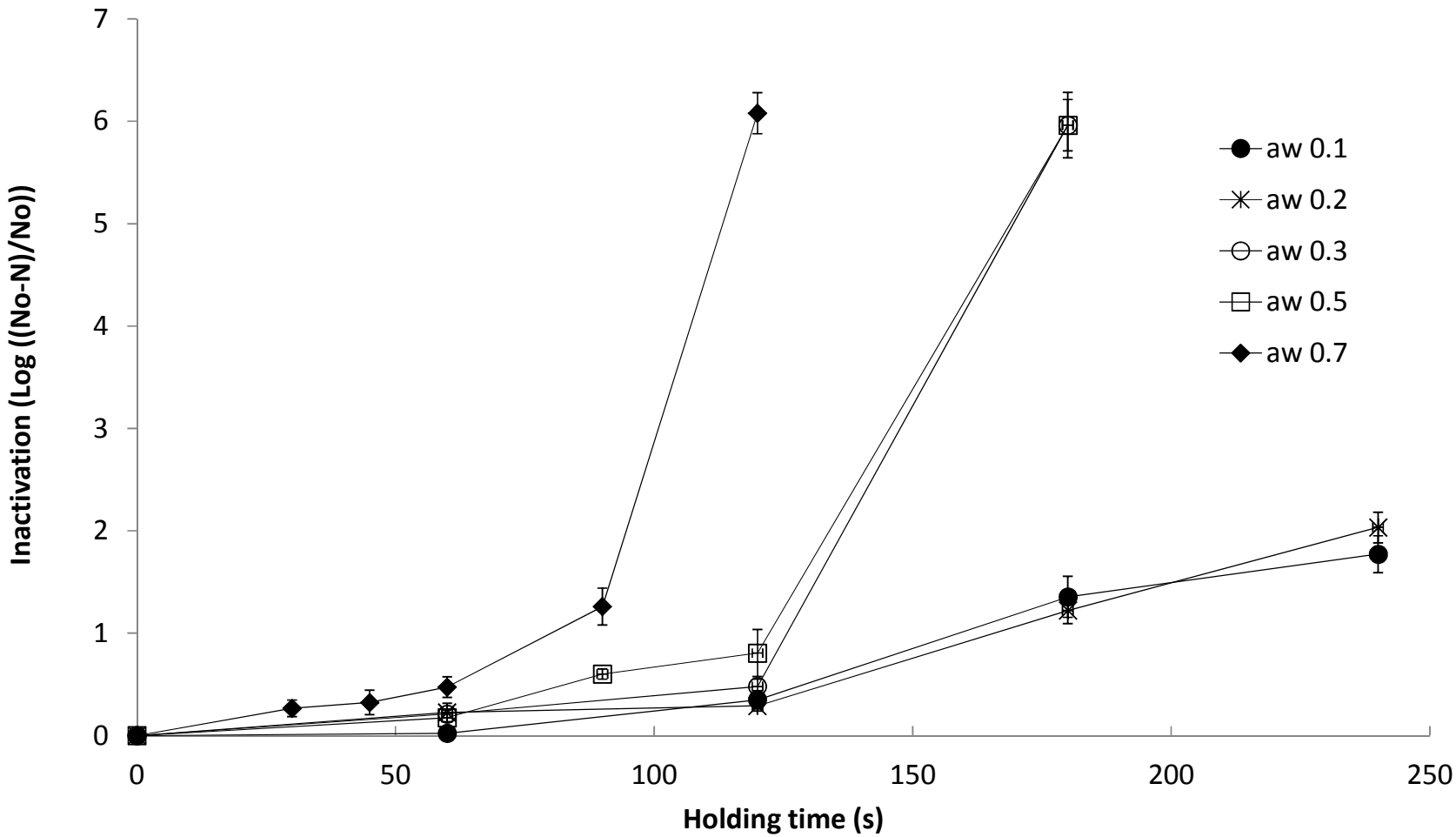

Inactivation (Log ((N$_o$-N)/N$_o$)
Holding time (s)
a$_w$ 0.1


Inactivation (Log ((N$_o$-N)/N$_o$)
Holding time (s)
a$_w$ 0.2


Inactivation (Log ((N$_o$-N)/N$_o$)
Holding time (s)
a$_w$ 0.3


Inactivation (Log ((N$_o$-N)/N$_o$)
Holding time (s)
a$_w$ 0.5


Inactivation (Log ((N$_o$-N)/N$_o$)
Holding time (s)
a$_w$ 0.7


Inactivation (Log ((No-N)/No))
Holding time (s)
0
1
2
3
4
5
6
7
0
50
100
150
200
250
aw 0.1
aw 0.2
aw 0.3
aw 0.5
aw 0.7

a_w	Glycerol (g/100g solution)
0.10	96
0.20	92
0.30	88
0.40	84
0.50	77
0.60	72
0.70	64

a_w	D (min)
0.10	1.7
0.20	1.0
0.30	0.7
0.50	0.8
0.70	0.5