SURVIVAL AND DEVELOPMENT OF *STAPHYLOCOCCUS* IN EGG PRODUCTS

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ABSTRACT

In this work, *Staphylococcus* has been employed to simulate a contamination of different egg foodstuffs. Liquid (egg white, egg yolk and whole egg) and also solid (Spanish potato omelette) products have been employed as model foods. The effect of different parameters, i.e. oxygen availability, cooking temperature and storage temperature, on microorganism survival and development has been evaluated. In addition, the structure of solid foods has been analysed by means of rheological measurements.

Results showed that *Staphylococcus* behaviour in liquid media was strongly influenced by the oxygen concentration, which is determinant for the specific growth rates (μ). In solid foods, the increase of cooking temperature to 100ºC reduced the microbial viability by one order of magnitude with respect to the raw foodstuff. Additionally, it was observed that the structure of the omelette, which depended on the cooking temperature, was a key parameter regarding the μ values. Therefore, to avoid the risk of food poisoning by *Staphylococcus* proliferation, in the case of egg products cooked only for few minutes, the cooking temperature should be higher than 100ºC and

the subsequent storage should be at refrigeration temperatures that increase the lag phase and decrease the growth rate.

Key words: egg products; food safety; omelette; rheology; *Staphylococcus*.

1. INTRODUCTION

Hen eggs are used as an economical source of high quality proteins commercialised in the form of shell eggs, but also liquid, frozen, and dried products (Muñoz et al., 2015; Upadhyaya et al., 2017). In addition, as one of the most versatile products, eggs are widely used in food industries on account of its functional attributes such as foaming, emulsifying, gelling, colouring and flavouring properties (Lechevalier et al., 2017).

World egg production and consumption have been increasing for the past decades (Abín et al., 2018), and current issues, such as food safety, should be considered in modern egg production (Wang et al., 2017a). Shell eggs can be contaminated with many types of microorganisms (Neira et al., 2017), including pathogens, and, thus, they present a risk for the transmission of foodborne disease to consumers (Al-Ajeeli et al., 2016). In fact, consumption of eggs and egg products has been often linked to food poisoning outbreaks due to their contamination with pathogenic bacteria (Moyle et al., 2016; Muñoz et al., 2015). In Spain, almost 40% of the outbreaks notified are related to consumption of eggs or derivative products. Thus, this is the most significant sector within food industry to study regarding the prevention of foodborne diseases.

Microbiological quality and safety of egg and egg products is largely subject to adequate cooking, handling, cooling and storing. It is well known that cooking temperature is a key factor regarding egg and egg derivatives safety. Indeed, it is

recommended that eggs should be cooked until the whites and yolks are coagulated and, in case of dishes containing fresh eggs a temperature of 75 ºC should be reached (R.D. 1254/1991). Nevertheless, in many households and even in many eating establishments, egg products are consumed without being totally cooked as, for example, soft-boiled eggs or "runny" omelettes, which increases food poisoning risks.

Epidemiological studies show that eggs are important sources for consumers' exposure to pathogens. Specifically, *Salmonella* and *Campylobacter* have received much attention and the incidence of these genera in eggs and eggs products has been thoroughly analysed (Alter, 2017; Gast and Jones, 2017; Jonaidi-Jafari et la., 2016; Kaldhone et al., 2017; Martelli et al., 2017; Messelhäusser et al., 2011). Additionally, some attempts have been carried out to study the behaviour in egg products of microorganism such as *Bacillus*, *Serratia*, *Staphylococcus* or *Pseudomonas* (Ananou et al., 2018; De Reu et al., 2006). *Staphylococcus* spp. is well known to produce a wide variety of toxins and it has also been described as a genus with ability to form amines, which, due to its toxicological characteristics, are responsible of outbreaks of food poisoning (Wang et al., 2017b). So, the prevention of *Staphylococcus* proliferation is an issue of great interest. However, few attention has been paid in literature to *Staphylococcus* in relation with egg products and, as far as we know, none work has been carried out in solid egg foodstuffs.

Hence, in the present work, *Staphylococcus* has been employed to simulate a contamination of liquid and solid egg products. The evolution of this bacteria has been monitored to evaluate the effect of different conditions of cooking and storage. In addition, the effect of oxygen availability has also been analysed. In all cases the specific growth rate (μ) values were obtained and compared in order to evaluate the *Staphylococcus* behaviour. Finally, the structure of the model omelette was evaluated by

means of rheological measurements with the aim to identify possible effects of structure on microorganism growth.

2. MATERIALS AND METHODS

2.1 Microorganism

Staphylococcus warneri (CECT 236) acquired from the Spanish Collection of Type Cultures was employed as model bacterium.

2.2 Culture media and experimental conditions

Shell eggs and potatoes were purchased at a local supermarket.

2.2.1 Liquid model food: white-, yolk- and egg- medium

For preparing the medium, egg white, egg yolk or whole egg was diluted in distilled water in sterile conditions (10% v/v). The inoculum was prepared by transferring a loopful of refrigerated working cultures on Petri dishes to 500 mL Erlenmeyer flasks containing 100 mL of each medium.

The white- and yolk- medium were incubated under aerobic conditions (250 rpm), whereas three types of experimental conditions were assayed for the whole egg-medium:

• Aerobic conditions (7.8-8.2 mg/L dissolved oxygen): 0.5 L Erlenmeyer flasks containing 100 ml of inoculated medium were incubated at 250 rpm.

• Hypoxic conditions (4.1-6.9 mg/L dissolved oxygen): 100 mL full bottles closed with screw tops were cultured without shaking.

• Anoxic conditions (<1 mg/L dissolved oxygen): the conditions described for hypoxic conditions were also employed, but, in this case, the initial dissolved oxygen was removed from the medium by flushing sterile nitrogen.

101 The white- and yolk- medium were incubated under three different temperatures
102 (11, 25 and 37ºC), whereas egg-medium experiments were carried out at 20 ºC.
103 The growth of *Staphylococcus* was determined by plating samples taken at different
104 times on Nutrient Broth Agar (Biokar).

105 **2.2.2 Structured model food: Spanish potato omelette**

106 The preinoculum was prepared from a refrigerated stock of Petri dishes by
107 transferring a loopful of the cultures to 500 mL Erlenmeyer flasks containing 100 mL of
108 Nutrient Broth. After 24 hours of incubation, 2 mL of this culture were centrifuged
109 (13000 rpm, 5 min) and the pellet was resuspended in saline solution (NaCl 0.9% v/v)
110 and centrifuged again. The raw omelette medium was prepared in sterile conditions by
111 stomaching the preinoculum pellet resuspended in 20 mL of whole liquid egg and 30 g
112 of small boiled-potato pieces. The initial concentration of microorganism was
113 approximately 10^7 CFU/g. Sterile 12 mL syringe-bodies (1.5 cm in diameter and 7.6 cm
114 in length) were packed with this mixture, reaching inside 4 cm in height. Then, the
115 syringes were closed by placing sterile aluminium foil sealed with Teflon® at the
116 syringe tip and a cotton wool plug on the top (Noriega et al., 2010a). Syringes so
117 prepared were placed during 8 min in an oven at different temperatures (60, 80 and
118 100ºC) to simulate the omelette cooking. Finally, the syringes were incubated at
119 different temperatures (6, 20 and 30ºC) to simulate different storage conditions. The
120 employment of syringes as containers mimics the environmental conditions at the
121 surface and different depths of a real omelette.

122 Sampling was carried out by taking 1 g of the model omelette at different
123 longitudinal positions: 3.7-4.0 cm (surface), 2.0-2.3 cm (middle) and 0.0-0.3 cm
124 (bottom) (Figure 1). All samples were taken in triplicate. Each sample was transferred
125 to a stomacher bag and homogenized with 9 mL of sterile saline solution and after that

serial decimal dilutions of the mixture were plated in triplicate onto Nutrient Broth Agar and incubated at 30 °C for 48 h before counting.

2.3 Characterization of the structured media

2.3.1 Reometry

Rheological measurements were carried out employing a Haake MARS II rotational rheometer. A plate/plate measuring system (PP60Ti) with a gap of 1 mm was used. All tests were carried out at 20 ± 0.1 ºC. Before measuring, samples rested for at least 15 min to allow the stresses induced during sample loading to relax (Laca et al., 2010a). The frequency sweeps were carried out from 0.1 to 10 Hz at a constant shear stress of 5 Pa.

2.3.2 Microscopy

The model omelettes were observed after inoculation by optical microscopy (Olympus BX61).

3. RESULTS AND DISCUSSION

3.1 Liquid model foods: white-, yolk- and egg - medium

3.1.1 Effect of composition

In Figure 2 it can be observed the behaviour of *S. warneri* in liquid yolk- and white-medium at 37ºC. It should be noticed that there was no growth in white-medium at the optimum temperature of the bacteria (37 ºC). Indeed, the viability decreased from the first hours and, after 25 hours, viable microorganisms were not detected in the medium. This can be explained due to the characteristic composition of egg white, since albumen proteins, mainly lysozyme and ovotransferrin, are well known to play important antimicrobial roles. Specifically, Bedrani et al. (2013) found that these both proteins

exhibit antimicrobial activity against *S. aureus*. Furthermore, several additional minor proteins and peptides have also been reported as potential protectors against bacterial contamination (Bedrani et al., 2013; Baron et al., 2016).

Figure 3 shows the behaviour of *S. warneri* in the medium prepared with whole egg (yolk and albumen) at different oxygen concentrations at 20ºC. From Figures 2 and 3, it can be compared the growth of *Staphylococcus* in yolk- and in egg- medium in aerobic conditions and at room temperature (20-25 ºC). Although in both cases a notable growth is observed, the CFU increased in three orders of magnitude in the case of yolk (from $3x10^5$ to 10^8 CFU/mL), whereas in egg-medium the increment was only in two orders of magnitude (from $2x10^5$ to 10^7 CFU/mL). Other difference was, the lag phase that lasted approximately 3 h in the case of whole egg and 10 h in yolk-medium. The exponential phase was also very different in both cases, whereas a sharp slope was observed during the exponential growth of the bacteria in yolk-medium, the slope was much more moderate in egg-medium. Indeed, this is clearly reflected by the specific growth rate values: 0.3172 h^{-1} and 0.0837 h^{-1} for yolk- and egg- medium, respectively (Table 1). Additionally, the necessary time to achieve the stationary phase of growth was shorter for yolk-medium (~15 h) than for egg-media (~50 h).

3.1.2 Effect of temperature

Yolk resulted an accurate medium for *S. warneri* survival at all the assayed temperatures. At 11 ºC the culture maintained its viability, however the cell growth was negligible and the microorganism concentrations remained around $3x10^5$ CFU/ml during the 3 days that lasted the experiment. On the contrary, bacteria increased approximately in three orders of magnitude when the incubation was at 25ºC and at 37ºC. However, the differences of growth due to temperature can be easily observed when the specific growth rates are compared (Table 1). It was impossible to obtain μ

from the experiment at 11ºC since no exponential phase was found. Nevertheless, the values obtained at 25 and 37 ºC were 0.2810 and 0.3172 h^{-1}, respectively, which clearly indicates that the rate of growth was higher for higher temperature. Furthermore, the lag phase was also different depending on temperature, it lasts 10 h at 25ºC, whereas there was non-existent at 37ºC.

3.1.3 Effect of oxygen concentration

Due to its relatively low solubility in water, oxygen is the substrate most likely to limit microbial growth in liquid products, but especially in solid foods, where the presence of diffusional limitations plays an important role. Different works about the conditions and mechanisms that govern the growth, survival and proliferation of facultative bacteria, such as *Listeria*, in low O_2 food environments have been carried out (Lungu et al., 2009; Noriega et al., 2008; Noriega et al., 2010a). To the best of our knowledge, it is remarkable that no works were previously developed regarding the genus *Staphylococcus*. So, in order to know the effect of oxygen concentration on this bacteria development, *S. warneri* growth was monitored in liquid medium with different concentrations of dissolved oxygen. For these experiments, the liquid medium prepared with whole egg was employed as model of real egg derivatives. As can be observed in Figure 2, although *Staphylococcus* grew under all assayed aeration levels, the level of growth depended on the oxygen concentration. Specifically, the maximum concentration of bacteria achieved in anoxic conditions was $6.7x10^5$ CFU/ml, whereas in hypoxic and aerobic conditions the maximum concentrations were $2.0x10^6$ and $1.4x10^7$ CFU/ml, respectively. The effect of oxygen can be also easily noticed considering the specific growth rate at the exponential phase values, since μ in aerobic conditions is twice the value obtained in hypoxia and it is four times higher than in anoxic conditions (Table 1).

3.2. Structured model food: Spanish potato omelette

3.2.1. Structure of the model food

In the micrographs of the potato omelette no differences could be appreciated between the structure of cooked and non-cooked samples, so in Figure 4 it is only shown as an example an image of a non-cooked omelette. Potato cells and also air bubbles originated by the foaming properties of egg can be easily observed. Additionally, higher magnification allowed the observation of isolate coccus of *Staphylococcus* immersed within the liquid egg (see arrows in Figure 4B).

The experimental data of all frequency sweep tests were correlated to the following power law equation, usually employed to characterise weak gel foods (Gabriele et al., 2001; Laca et al., 2010b; Rodil et al., 2017):

$$G^* = A \cdot \nu^{1/z}$$

where G^* is the complex modulus in Pa, ν the frequency in Hz, z (dimensionless) the coordination number and A (G^* in Pa at 1 Hz) the proportional coefficient. The coordination number (z) is a measure of the number of rheological units correlated with one another in the three-dimensional structure, whereas the proportional coefficient (A) is related to the strength of the interaction between those units (Mancini et al. 2002).

It can be noted that values found here for parameters A and z of omelette samples were in the same order of magnitude of those reported by Ndayishimiye et al. (2016) for sweet potato-wheat doughs (A: 7.2-8.9 kPa $s^{1/z}$ and z: 5.2-5.7) and were within the range described by Migliori et al. (2009) for Yorkshire pudding batter prepared with different egg amounts (A: 2.5-31 kPa $s^{1/z}$ and z: 2.5-28) (Table 2). When results obtained for the different samples analysed in the present work are compared, the

rheological parameters indicate the existence of some structural differences between cooked and non-cooked omelettes. Regarding the network extension, *z* maintained a similar value for the different samples, except for the sample cooked at 100 ºC. With respect to the network strength, a clear trend can be observed, since the *A* value raised when cooking temperature increased. Hence, certain differences could be appreciated between the structure of non-cooked samples and omelettes cooked at 60 ºC and 80 ºC, and the differences became more marked for the omelette cooked at higher temperature. Certainly, parameters *A* and *z* reflect high structural modifications when the sample was cooked at 100 ºC. Specifically, this cooking temperature slightly decreased the number of interacting units in the three-dimensional structure and, at the same time, notably increased the strength of these interactions. This effect can be explained by considering the temperature-induced transitions due to starch and egg gelatinization acting as material network strengthening factors.

3.2.2. Survival of *Staphylococcus* after cooking

The United States Department of Agriculture (USDA) provides minimum temperatures and holding times required to accurately treat liquid egg products. Specifically, whole egg should be treated at 60 ºC for 2.5 min (Froning et al., 2002). However, not only the setpoint temperature and time have an important effect on thermal inactivation rates of food microorganisms, but other aspects of heating treatment conditions, i.e. heating rate and heating uniformity, are also determinants (Kou et al., 2018). In the present work, it is remarkable that the cooking process carried out for 8 min at 60ºC and 80ºC practically did not affected the number of viable microorganisms in the omelettes, maintaining the inoculation level (~ 10^7 CFU/g). It should be pointed out that omelettes are a solid product and the recommendations for egg products pasteurization usually refers to liquid foodstuffs where the convention

252 plays an important role in the heat transfer. On the contrary, when the omelette was
253 cooked at 100ºC a reduction of one order of magnitude was achieved decreasing the
254 concentration of viable *Staphylococcus* from $1.8x10^7$ to $2.0x10^6$ CFU/g (Figure 5).
255 According to the Spanish Government, "in all catering business those foodstuffs
256 elaborated with fresh eggs should be cooked at least at 75ºC" (R.D. 1254/1991). Thus,
257 temperatures higher than 75 ºC are recommended in order to assure that 75ºC are
258 achieved at the internal area of the product, especially if the cooking time is short.
259 Despite this regulation, in some European countries, including Spain, it is a usual
260 practice that egg products are consumed without being totally cooked (soft-boiled eggs,
261 "runny" omelettes…) not only at households, but also at restaurants and canteens,
262 increasing food poisoning risks.

263 **3.2.3. Development of *Staphylococcus* during storage**

264 A frequent custom in households and also in eating establishments is to store the
265 Spanish potato at room temperature. Simulating a contamination by *Staphylococcus*, in
266 Figure 4 it is shown the bacterial growth at 20ºC at different longitudinal positions in
267 model omelettes cooked at different temperatures (60, 80 and 100 ºC). The highest
268 bacterial concentration was achieved in the surface for the omelette cooked at 60 ºC
269 with an increase of one order of magnitude. The middle and bottom positions show
270 identical behaviour with a growth slightly lower. Qualitatively, similar behaviours can
271 be observed in the three positions analysed of samples cooked at 80 and 100 ºC.
272 Nevertheless, when the lag phase and the specific growth rates are analysed in detail,
273 some differences can be observed (Table 1). It is remarkable that in the three omelettes
274 cooked at different temperature there was not detected a lag phase in the samples taken
275 from the surface, whereas a lag phase of approximately 24 h was observed in the inner
276 positions. It seems that in this solid media, the absence of oxygen provoked the

277 enlargement of this lag phase. With regards to the specific growth rates, similar values
278 were found for each cooking temperature independently of the longitudinal position.
279 Comparing the μ obtained for the different omelettes, those cooked at 60 and 80 ºC
280 showed similar values (0.026-0.029 h^{-1}), quite close to those found in liquid medium
281 under anoxic conditions (0.024 h^{-1}). This indicates that, although the surface is in
282 contact with air, and this higher availability of oxygen makes cell growth starts more
283 quickly, diffusional limitations exist even in the surface position (thickness 0.3 cm)
284 contributing to a slower bacterial growth.

285 Other aspect to take into account is the confinement of the bacteria inside a
286 structured media, which can also have influence on cell growth rate (Noriega et al.,
287 2010b). In this sense, the omelette treated at 100 ºC exhibited lower values of the
288 specific growth rate (0.014-0.017 h^{-1}). This seems to corroborate the results found by
289 rheological measurements, which indicated some changes on the structure of the
290 omelette treated at 100ºC. Aspridou et al. (2014) studied the effect of the microstructure
291 of the medium on the growth of *Listeria monocytogenes*. They reported that the growth
292 of the pathogen was faster in the liquid than in the gelled systems. In a similar manner,
293 here, the growth of *S. warneri* was faster in liquid medium in comparison with the
294 omelette, which is clearly affected by the network formed between egg proteins and
295 starch.

296 With the aim to evaluate the effect of storage temperature when an accidental
297 contamination with *Staphylococcus* takes place, model omelettes inoculated with *S.*
298 *warneri* and cooked at 60 ºC during 8 min have been incubated at refrigerated
299 conditions (6 ºC) and room temperatures (20 and 30 ºC). As can be observed in Figure
300 6, the growth at refrigerated temperature (6 ºC) was very slow, being similar in all the
301 positions studied and in the same order of magnitude as the initial concentration. At

simulated room temperatures (20ºC and 30ºC) the cell growth were higher in all the positions, achieving values around 10^8 CFU/mL in 72 h. Clear differences can be observed with respect to the lag phase that was shorter for higher temperatures and oxygen availability. Indeed, at 6 ºC the microorganism showed a lag-phase of 24 h in the surface, whereas at middle and bottom positions it lasted 48 hours. At 20ºC there was no lag-phase in the surface and it lasted 24 hours at middle and bottom positions, whereas at 30 ºC lag-phase was not observed for any of the analysed positions.

As expected, the specific growth rates were very different depending on the storage temperature. So, the ranges for μ values were 0.018-0.022 h^{-1}, 0.028-0.029 h^{-1} and 0.034-0.046 h^{-1} for 6, 20 and 30 ºC, respectively. Differences for the longitudinal position only were detected for the highest temperature, where the growth was faster on the surface. As explained in previous works (Noriega et al., 2008; Noriega et al., 2010a), the reason is that just on the surface there is no problem with the availability of oxygen, whereas in the inner positions diffusional limitations exits, which makes the bacterium grow slower. Since the absence of diffusional limitations only occurs in the very narrow layer close to the surface and the layer analysed had around 0.3 cm of thickness, the preferential growth on the surface only could be observed when the effect of oxygen availability on bacterial growth was more marked, i.e. at 30 ºC.

It is obvious that the long duration of the lag-phase and the lower μ value at refrigerated temperatures entailed a lower growth of *Staphylococcus* in comparison to room temperatures. In this context, the Spanish Government (R.D. 1254/1991) established that the foodstuffs which included fresh eggs as ingredient must be consumed within the following 24 hours to their elaboration and they must be preserved at 8ºC until their consumption. With this regards, it should be noticed that in 24 hours

326 the growth of the bacteria is negligible in all the studied positions at 6 ºC of storage
327 (Figure 6).

328 So, regarding storage conditions, it can be concluded that the lack of oxygen is
329 not an effective measure to avoid the growth of *Staphylococcus* genus. On the contrary,
330 refrigeration is determinant to reduce the growth rates of microorganisms. Thus, the
331 storage of egg and egg products refrigerated is key to reduce food poisoning risk, not
332 only at household level, but also at restaurant, hotel and catering sectors.

333

334 **4. CONCLUSIONS**

335

336 *S. warneri* was not able to growth in a liquid medium mainly composed of egg
337 white due to the antimicrobial effects of albumen proteins. On the contrary, yolk
338 showed to be an accurate substrate for the development of the bacteria. When a mixture
339 of yolk and albumen was employed as liquid media, it was proved that *Staphylococcus*
340 growth was strongly favoured by the amount of dissolved oxygen which was clearly
341 reflected by the specific growth rates at the exponential phase.

342 In solid foods (i.e. model potato omelette), it was found that the cooking at
343 100ºC during 8 min achieved a reduction of one order of magnitude in the concentration
344 of bacteria, whereas cooking at lower temperatures did not affect bacterial viability. In
345 addition, the structure of omelettes cooked at 100ºC changed, as it was reflected by
346 rheological measurements. This structural change exerted a great influence on the
347 specific growth rates of *Staphylococcus* that were lower than half the value obtained
348 with lower cooking temperatures. While anaerobic conditions are not an effective
349 barrier against the growth of *Staphylococcus*, refrigerate temperature of storage is a
350 determinant measure to take into account in order to avoid food outbreaks originated by

this genus. Thus, from a practical perspective it results essential that, in any environment (household, catering industry) egg products should not be stored at room temperatures even for a few hours.

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Table 1. Specific growth rate (μ) values at the exponential phase of growth, obtained from the different conditions assayed. In all cases CV < 5% and $r^2 \geq 0.91$.

Culture medium	Longitudinal position	Incubation conditions	Lag phase (h)	μ (h^{-1})
Liquid yolk	-	11 ºC / 250rpm	No growth was observed	
		25ºC / 250 rpm	10	0.3172
		37ºC / 250 rpm	-	0.2810
Liquid egg (yolk + albumen)	-	20 ºC / 250 rpm (aerobic)	3	0.0837
		20 ºC / 0 rpm (hypoxic)	3	0.0409
		20 ºC / 0 rpm (anoxic)	4	0.0237
Model omelette (cooked at 60ºC)	Surface	6 ºC	24	0.0176
	Middle		48	0.0220
	Bottom		48	0.0210
	Surface	20ºC	-	0.0282
	Middle		24	0.0289
	Bottom		24	0.0288
	Surface	30ºC	-	0.0461
	Middle		-	0.0338
	Bottom		-	0.0378
Model omelette (cooked at 80ºC)	Surface	20 ºC	-	0.0274
	Middle		24	0.0274
	Bottom		24	0.0262
Model omelette (cooked at 100ºC)	Surface	20 ºC	-	0.0176
	Middle		24	0.0143
	Bottom		24	0.0165

Table 2. Power-law parameters obtained from frequency sweeps. Average values ± SD are reported. In all cases $r^2 \geq 0.993$.

POTATO OMELETTE	Power-law parameters	
	A **(kPa $s^{1/z}$)**	***z***
Non-cooked	4.5 ± 0.3	11.4 ± 0.3
Cooked at 60°C	5.6 ± 1.8	11.3 ± 0.5
Cooked at 80ºC	5.9 ± 0.1	11.3 ± 0.3
Cooked at 100ºC	8.3 ± 0.2	10.8 ± 0.5

FIGURE CAPTIONS

Figure 1. Scheme of model omelette and sampling positions.

Figure 2. Growth of *S. warneri* in liquid: yolk- (full symbols) and albumen-medium (empty symbols) in aerobic conditions at different temperatures: 11ºC (circle), 25ºC (square) and 37ºC (triangle). In all cases CV >5%.

Figure 3. Growth of *S. warneri* in liquid egg-medium at 20ºC: Aerobic conditions (□), Hypoxic conditions (○) and Anoxic conditions (Δ). In all cases CV >5%.

Figure 4. Structured omelette micrographs. A: magnification 10x and B: magnification 100x.

Figure 5. Growth of *S. warneri* at 20ºC in model omelet at different longitudinal positions: A) surface, B) middle and C) bottom, cooked at different temperatures: 60 ºC (■), 80 ºC (▲) and 100 ºC (●). In all cases CV >5%.

Figure 6. Growth of *S. warneri* cooked at 60 ºC in model omelet at different longitudinal positions: A) surface, B) middle and C) bottom, incubated at different temperatures: 6 ºC (■), 20 ºC (▲) and 30 ºC (●). In all cases CV >5%.

Figure 1

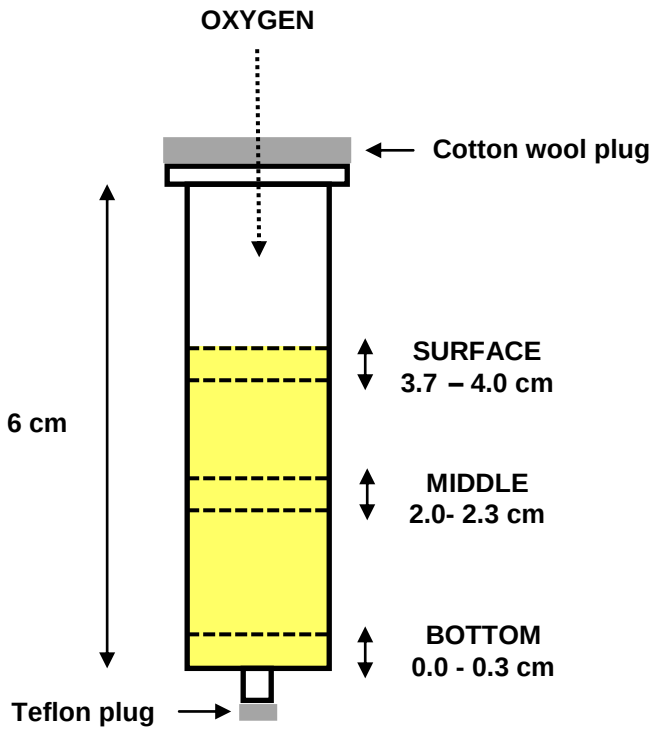

OXYGEN
Cotton wool plug
6 cm
SURFACE
3.7 – 4.0 cm
MIDDLE
2.0- 2.3 cm
BOTTOM
0.0 - 0.3 cm
Teflon plug

Figure 2

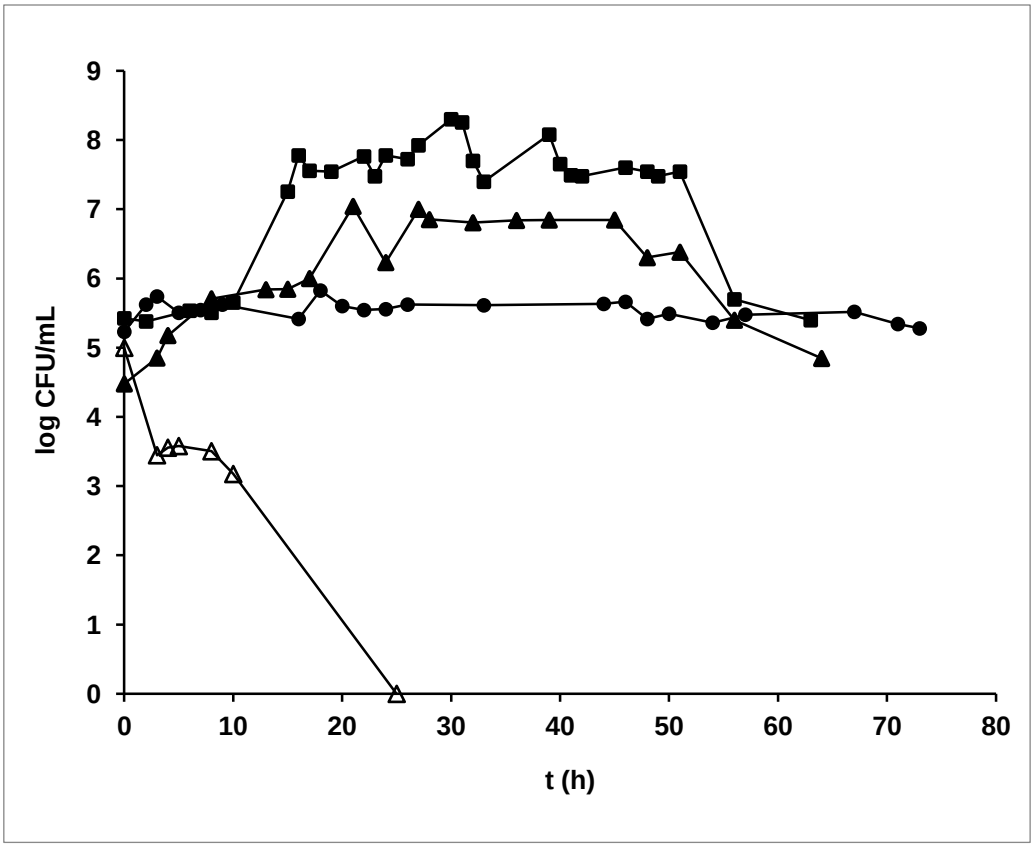


Figure 3

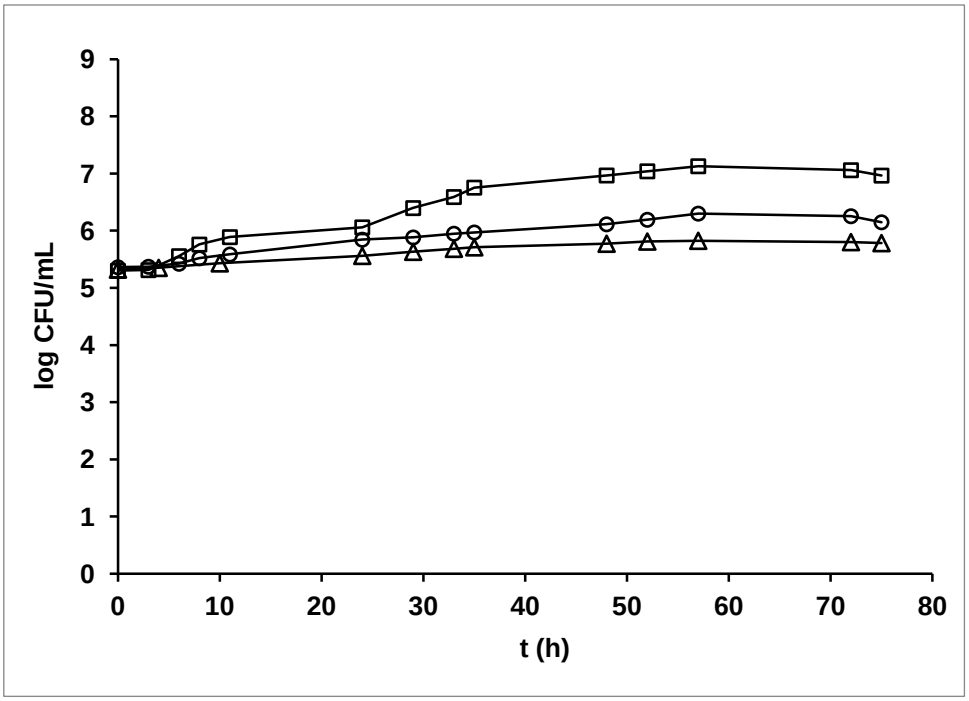


Figure 4

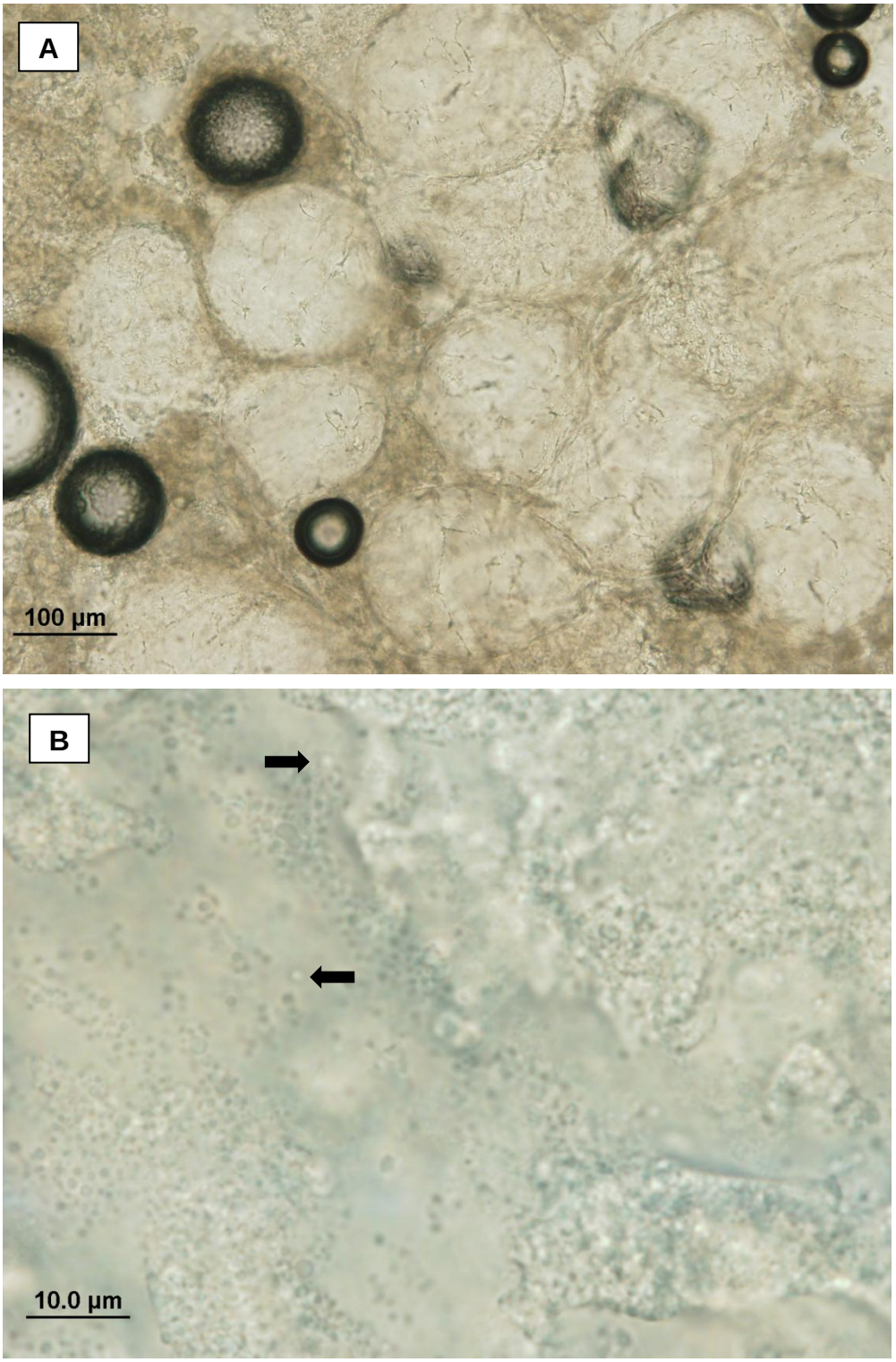
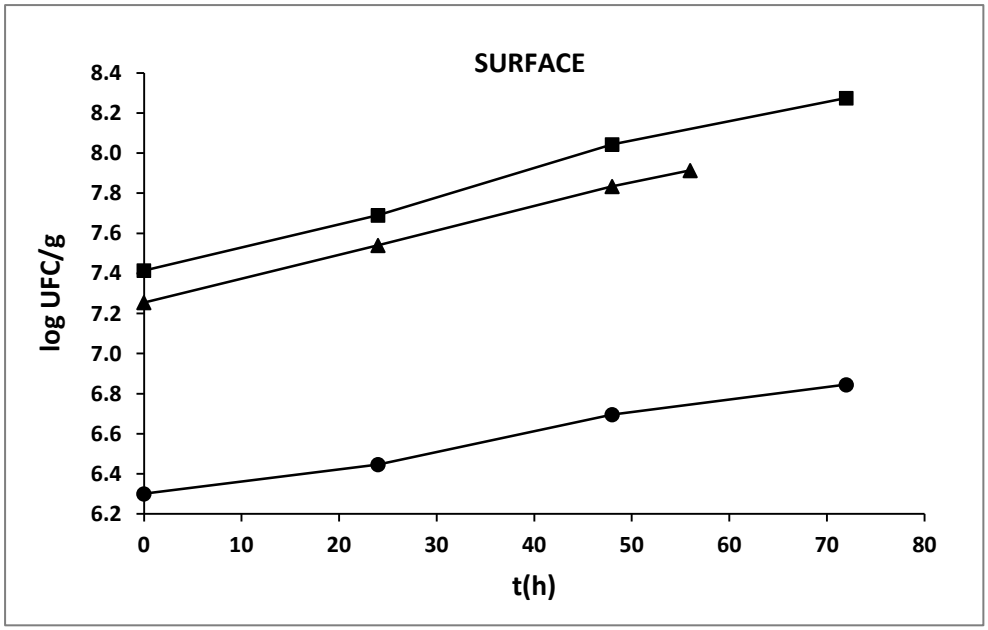
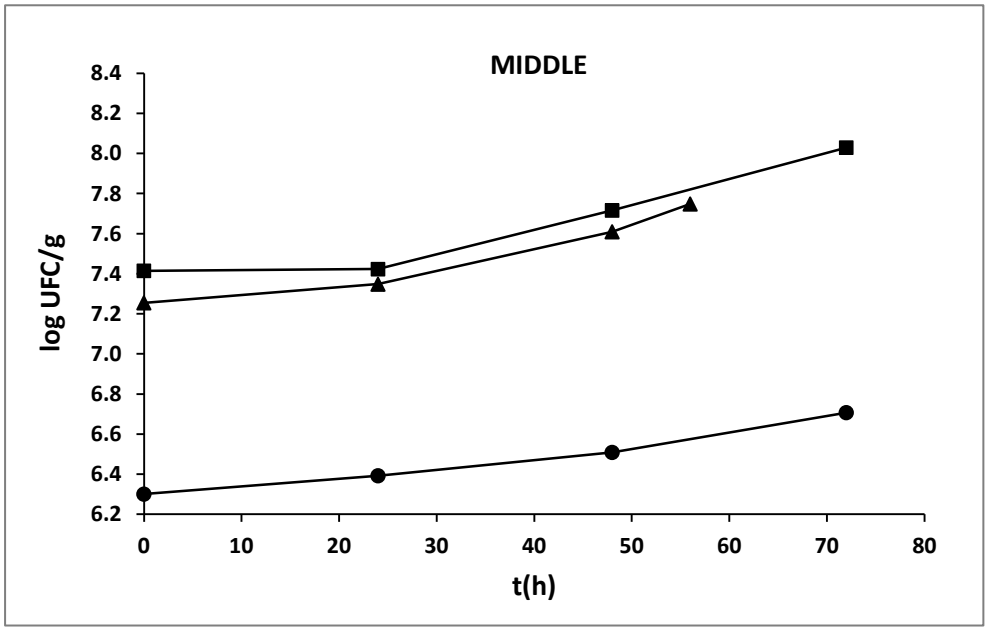


Figure 5

A)


SURFACE
log UFC/g
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
0
10
20
30
40
50
60
70
80
t(h)

B)


MIDDLE
log UFC/g
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
0
10
20
30
40
50
60
70
80
t(h)

C)

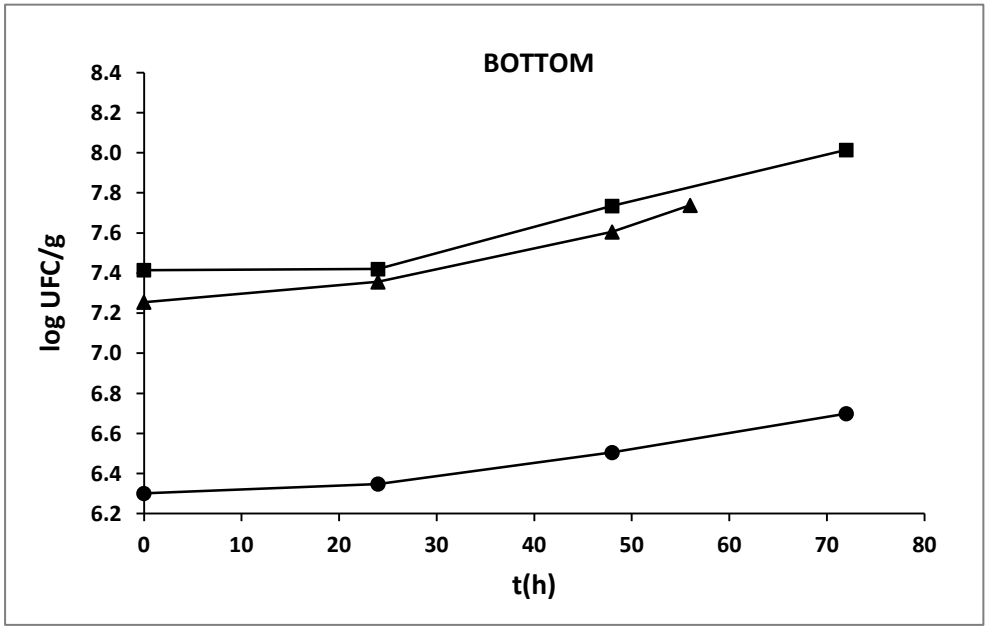
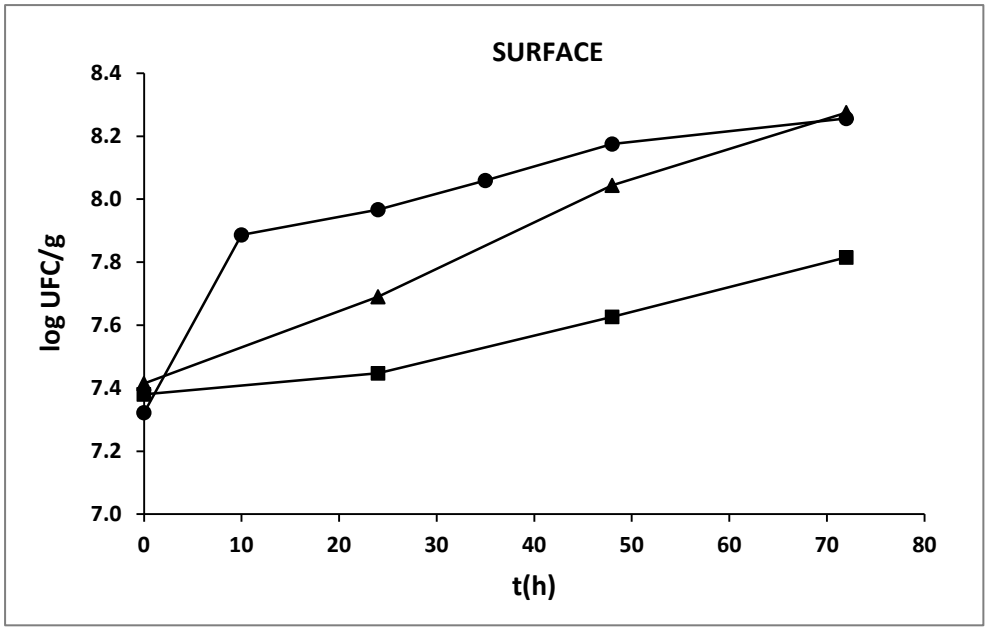
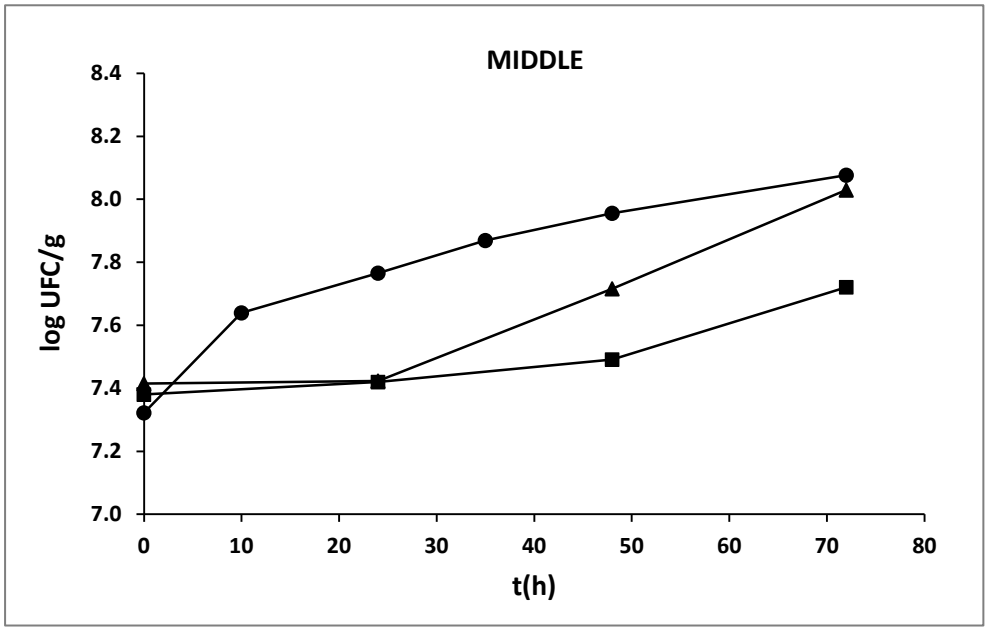

BOTTOM
log UFC/g
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
0
10
20
30
40
50
60
70
80
t(h)

Figure 6

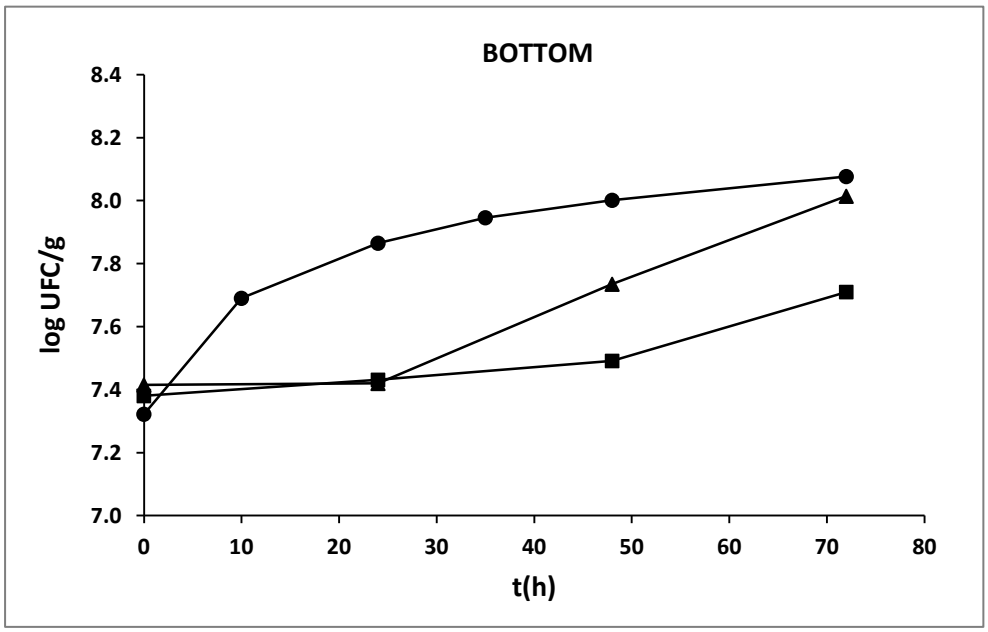
A)


SURFACE
log UFC/g
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
0
10
20
30
40
50
60
70
80
t(h)

B)


MIDDLE
log UFC/g
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
0
10
20
30
40
50
60
70
80
t(h)

C)


BOTTOM
log UFC/g
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
0
10
20
30
40
50
60
70
80
t(h)