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COMPARATIVE ANALYSIS OF VITRIFICATION TECHNIQUES WITH SLUSH NITROGEN AND LIQUID NITROGEN USING NUMERIC SIMULATIONS (CFD)

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Abstract. *Cryopreservation is a prominent area that studies the different ways of preserving biological material. In this work, we seek to compare the performance of slush nitrogen in relation to liquid nitrogen as a cooling fluid for vitrification processes. Slush nitrogen is a subcooled mixture of liquid nitrogen containing solid nitrogen particles suspended in liquid. Slush nitrogen is considered a potential substitute for liquid nitrogen in cryogenic processes, thanks to its high density and high heat capacity. The evaporation of slush nitrogen (SN2) and liquid nitrogen (LN2) will be analyzed, as well as the effect that each fluid has on the solidification time and the water-cooling rate. This analysis was performed using a numerical modeling (CFD) in ANSYS Fluent ® software. With the simulation, it was possible to observe that the slush nitrogen presents low vapor formation around the sample when compared to the liquid nitrogen, a preponderant factor to reach high cooling rates, above 1500 K/min. This aspect provided a short water solidification time, approximately 3s.*

Keywords: *cryopreservation, vitrification, slush nitrogen*

1. INTRODUCTION

In biology, the cryopreservation studies the low temperatures effect in the cells, tissues and living organisms, aiming to preserve the composition of cells for longs periods. These are techniques of high complexity that are constantly improving due to their wide clinical applicability. Several studies have been conducted so that these procedures can offer good results in the process of freezing and thawing human ovarian tissue, sperm, embryos and other biological materials. Conceptually, cryopreservation is a procedure by which cells are suspended in a solution of salts and a low-molecular-weight organic compound, cooled to very low subzero temperatures, usually – 196 ºC in liquid nitrogen, and stored for long period, then warmed and recovered to resume their normal function (Leibo and Pool, 2011).

Cryopreservation protocols have been developed to prevent or to control intra and extracellular ice formation during freezing. Determining an appropriate protocol is a critical step for sample survival. This success depends on some factors, such as the nature of the cellular material, type and concentration of cryoprotectant, and selection of an adequate cooling rate for the process.

Currently, cryopreservation processes use two freezing techniques, slow freezing and vitrification (ultra-fast freezing). Both differ in the speed of freezing and in the level of concentration of cryoprotectant used in the process. The slow freezing method is a conventional process of cryopreservation, characterized by very low cooling rates and concentrations of cryoprotectants, generally between 1 to 1000 ºC/min and 1 to 1.5 M, respectively (Shaw and Johns, 2003). The vitrification process is an alternative to the conventional method of slow freezing due to the high cooling rates associated with the procedure (Saragusty and Arav, 2011). The ultra-fast freezing process confers a greater probability of survival of the biological material (H, due to the need for low concentrations of cryoprotectants used in the procedure, thus reducing the risk of toxicity on the samples (Lee *et al*., 2010), and avoiding also to the formation of ice crystals that can cause damage to the biological material.

To achieve the ultra-fast freezing, several studies and methodology were developed, including the OPS (Open Pulled Straw). The OPS method was developed by Vajta *et al*. (1998) and consists of collect the biological material (embryos) through a tube by the capillarity effect and then submerge them in a container filled with liquid nitrogen, until the suspended cells inside the tube are completely frozen. Through the OPS method, cooling speeds above 20,000 ºC/min can be achieved if compared to the 10 ºC/min, on average, observed in slow freezing, with a short contact with cryoprotective agents, minimizing the toxic and osmotic effects of cryoprotective substances, according shown in Fig. 1.

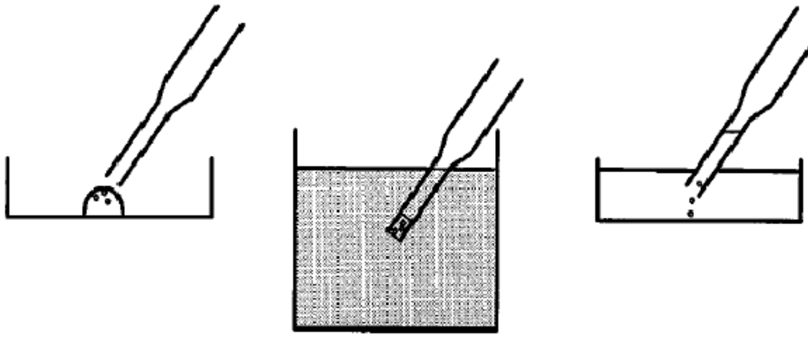

Figure 1. Open Pulled Straw method (Vajta et al. 1998)

The main obstacle to achieving higher cooling rates in OPS method, is associated with the Leidenfrost effect that occurs during the pool boiling, due to the large temperature difference. The formation of vapor bubbles around the outer surface of the sample causes a drastic drop in the heat transfer coefficient between the surface and the refrigerant (straw - liquid nitrogen), since the vapor film that forms on the surface works as a thermal insulator, hindering the heat transfer process (Su *et al.*, 2017). According to Nowshari and Brem (2001), with the removal of the steam film, it is possible to obtain high cooling rates in the vitrification processes.

The ideal cryopreservation protocol would combine the benefits of slow freezing with the benefits of vitrification. Following this path, Arav *et al.* (2002) presented a methodology capable of minimizing the formation of steam blanket around the sample using slush nitrogen, a subcooled mixture of liquid nitrogen containing solid nitrogen particles.

This work aims to perform a numerical modeling comparing the application of slush nitrogen in the vitrification process of biological materials in relation to the use of liquid nitrogen. Calculations were performed by Computational Fluid Dynamics (CFD) code using ANSYS FLUENT software. All the simulations shown in this paper were performed for water, liquid (LN2) and slush nitrogen (SN2). In this paper are shown the analysis of the water solidification time, cooling rate and rate of vapor formation.

2. REMOVAL OF THE STEAM BLANKET IN VITRIFICATION PROCESSES

Several studies related to the removal of the vapor film around the sample have been developed in recent years. Different techniques were studied, and some showed promising results.

Jiao *et al.* (2006) numerically investigated the effects of the variation of the heat transfer coefficient during the cooling processes in ultra-thin straws for cell vitrification. To increase the heat transfer coefficient on the external surface, the straw was inserted into an oscillating heat pipe (OHP) that uses the pressure change to excite the movement of the vapor bubbles that form in the cavities of the OHP. This process allowed achieving ultra-high heat transfer coefficients, higher than 10^4 W/m^{2}K.

Another alternative presented by Su *et al.* (2017), consists of carrying out a thin film evaporation process. The process results from the injection of liquid nitrogen into the external surface of the evaporator that contains the biological material. Experimental results showed that a cooling rate of approximately 50.000 ºC/min was achieved in a temperature range from 10 ºC to -187 ºC. Thus, it was realized that the use of a thin film evaporation process that flows through the surface allows achieving high cooling rates, with relatively low concentrations of cryoprotectants.

Nowshari and Brem (2001), in their study with embryos, transformed liquid nitrogen into nitrogen slush, a two-phase cryogenic fluid with solid nitrogen particles suspended in liquid nitrogen, reaching an average temperature of 67 K by applying a low pressure inside a vessel containing liquid nitrogen. As a result, it was found that the main benefit of applying slush nitrogen instead of common liquid nitrogen is not derived from the temperature difference between the two systems itself, but mainly from the fact that SN2 minimizes the insulating vapor layer, a major factor in maximizing heat transfer in vitrification processes.

Arav *et al.* (2002), reduced the temperature of liquid nitrogen by applying a negative pressure in the chamber containing the LN2. As a result, they obtained slush nitrogen, a mixture of liquid nitrogen with solid nitrogen particles, with an average temperature close to 67 K. When applied as cooling fluid, it was found that the cooling rate average using slush nitrogen was dramatically higher when compared to liquid nitrogen. A typical device for producing slush nitrogen is shown in Fig. 2.

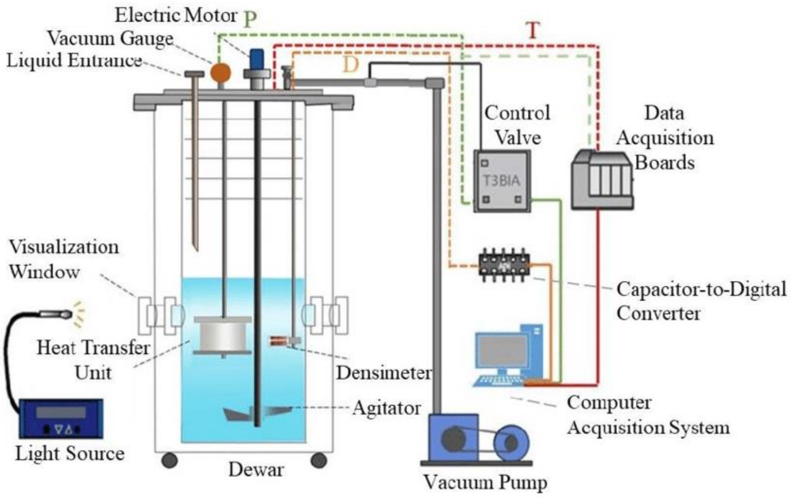

Figure 2. Experimental apparatus for production slush nitrogen (Wu *et al*., 2020)

In their experiments, Lee *et al.* (2010) used slush nitrogen instead liquid nitrogen in order to improve oocyte survival rate. Using low concentration of cryoprotectants such as propanediol and trehalose, they achieved up to 90% oocyte survival. The cooling rate obtained was 250.000 ºC/min. Therefore, an ultra-fast vitrification technique was developed for mouse oocytes that uses low concentrations of cryoprotectants and nitrogen slush in quartz capillaries, which combines the benefits of slow freezing and vitrification.

In recent years, slush nitrogen is considered a potential coolant for some HTS devices thanks to its high density and large heat capacity. Using slush nitrogen as coolant in HTS device can reduce the volume of cryogenic system and improve the heat transfer rate, a critical factor in vitrification processes (Wu *et al*., 2020).

3. MATERIALS AND METHODS

In this work, numerical simulations (CFD) using the commercial soft-ware ANSYS Fluent®, analyzing the cooling rate, solidification curve and vapor formation rate around at the sample.

3.1 Phisics modelling

A polypropylene straw, 30 mm long (L), 1.9 and 2.6 in internal and external diameter, respectively, was considered as a model. The straws were described as two concentric finite cylinders of different materials (fluid and solid). The straw is submerged in liquid nitrogen (case 1) and subsequently in slush nitrogen (case 2). The total straw volume is 0.5 µl, resulting in an L/D ratio of 13. This value is large enough to assume that the contribution of the axial heat flow is negligible. As a result, the system can be numerically solved as a one-dimensional heat conduction problem in an infinite cylinder (San Sinema *et al*. 2011). To reduce the computational cost, a symmetrical system was considered, assuming that, the physical phenomena that occur in 1/4 of the cylinder are like the other parts. The geometry and computational domain as shown in Fig. 3

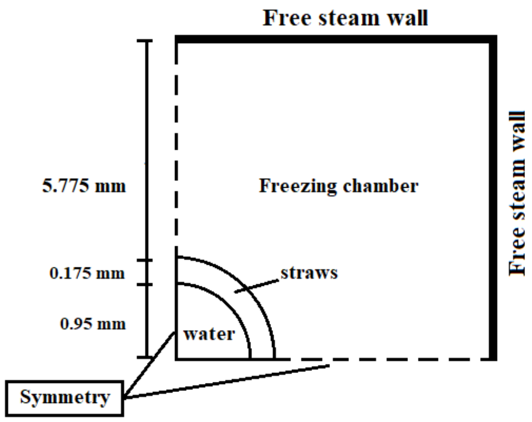

Figure 3. The geometry and computational domain

Due to the difficulty in obtaining thermal characteristics of cryoprotectants commonly used in vitrification processes, water will be used replacing the cryoprotectant. The heat transfer through the straw wall to the water (see Fig. 3a) was considered only by conduction. It was assumed that the water and the straw (polypropylene) are initially at the same temperature, 298 K. Initially the vessel (freezing chamber) is filled with liquid nitrogen at 77 K, for case 1, and slush nitrogen at 67 K, for simulation of the case 2, assuming without any traces of saturation. The vessel walls are considered infinite (see Fig. 3a) and are at the nitrogen temperature in each simulation. Although accurate calculations require thermal properties varying with temperature, in this case, the properties were assumed to be constant and determined by the temperature. Jiang and Zhang (2011) calculated the density of the nitrogen slush, with 22% of volumetric fraction of solids present in the mixture, is 885 kg/m^3, but in this work used the value described in Ishimoto *et al.* (2005). The other properties of slush nitrogen were considered making an approximation with the properties of liquid nitrogen at 67 K. The values are shown in Table 1.

Table 1. Estimated values of thermal properties of materials.

Material	Temperature, K	Density, kg/m^3	Thermal conductivity, W/mK	Specific heat, J/kgK	Viscosity, kg/ms	Surface tension
Polypropylene	-	900	0,22	1680	-	-
Water	298					0,072
Vapor nitrogen	77	4,51	0,007152	1122	0,000005428	0,008926
Liquid nitrogen	77	806	0.14581	2041	0.00016065	0,008926
Slush nitrogen	67	1028	0,1673	-	0,00266	0,01149

The pure solvent melting heat (enthalpy of fusion) of water is a constant value of 334 kJ/kg with the phase change temperature from liquid to solid around 273 K (0 ºC). In both cases, liquind and slush, simulations were performed in the normal condition of pressure and temperature (101.321 kPa and 298.15 K), so the saturation temperature of liquid nitrogen was regarded as 77 K.

3.2 Mathematical modelling

Commercial CFD software ANSYS Fluent was used to implement and solve the formulation of the film-boiling model as described in the following.

The numerical configuration for the liquid nitrogen-vapor phase change inside the chamber is performed using the implicit multiphase VOF (Volume of Fluid) model. For solidification of water, the SOLIDIFICATION and MELTING model was applied. The energy equation is shared between the phases. The VOF model treats energy as a mass-averaged variable, according to Eq. (1).

$$\frac{\partial}{\partial t}(\rho E) + \nabla \cdot \left(\vec{v}(\rho E + p)\right) = \nabla \cdot \left(k_{eff}\nabla T - \sum_q \sum_j h_{j,q}\vec{J}_{j,q} + \left(\bar{\bar{\tau}}_{eff} \cdot \vec{v}\right)\right), \tag{1}$$

The tracking of interfaces between phases is performed by solving a continuity equation for the volume fraction of each phase, as shown in Eq. (2).

$$\frac{1}{\rho_q}\left[\frac{\partial}{\partial t}\left(\alpha_q\rho_q\right) + \nabla \cdot \left(\alpha_q\rho_q\overrightarrow{v_q}\right) = \sum_{p=1}^{n}(\dot{m}_{pq} - \dot{m}_{qp})\right], \tag{2}$$

where ρ_q is the density of the *q* phase, α_q is the volume fraction of the *q* phase, $\overrightarrow{v_q}$ is the velocity of the *q* phase, $\dot{m}_{pq}$ is the mass transfer from the *p* phase to the *q* phase and $\dot{m}_{qp}$ is the mass transfer of the *q* phase to *p* phase.

A single moment equation is solved in the entire field, and the resulting velocity field as is mixed stages (vapor-liquid nitrogen), according to Eq. (3).

$$\frac{\partial}{\partial t}(\rho\vec{v}) + \nabla \cdot (\rho\vec{v}\vec{v}) = -\nabla_p + \nabla \cdot [\mu(\nabla\vec{v} + \nabla\vec{v}^T)] + \rho\vec{g} + \vec{F}, \tag{3}$$

where ρ is the density, $\vec{v}$ is the velocity, μ is the dynamic viscosity, $\vec{g}$ is the gravity acceleration and $\vec{F}$ is a force applied. The Lee equation for evaporation, which considers the evaporation of nitrogen, is represented by Eq. (4).

$$\frac{\partial}{\partial t}(\alpha_v\rho_v) + \nabla \cdot \left(\alpha_v\rho_v\vec{V}_v\right) = \dot{m}_{lv} - \dot{m}_{vl}\,, \tag{4}$$

where α_v is the volume fraction of vapor, ρ_v is the density of the steam, $\vec{V}_v$ is the velocity of the steam, $\dot{m}_{lv}$ is the mass transfer by evaporation and $\dot{m}_{vl}$ is the mass transfer by condensation.

The source term, S, is the is given by Eq. (5).

$$S = \frac{(1-\beta)^2}{(\beta^3+\varepsilon)} A_{mush}(\vec{v} - \vec{v}_p) , \tag{5}$$

where β is the volumetric fraction of liquid, ε is a small number to avoid divisions by zero, A_{mush} is the mush zone constant, $\vec{v}_p$ is the traction velocity of the solid, according to Eq. (6).

$$\begin{aligned} &\beta = 0 \; if \; T < T_{solidus}, \\ &\beta = 1 \; if \; T > T_{liquidus}, \\ &\beta = \frac{T-T_{solidus}}{T_{liquidus}-T_{solidus}} \; if \; T_{solidus} < T < T_{liquidus} , \end{aligned} \tag{6}$$

It was simulated cases considering a transient formulation during 4 s, with a time step size of 0.005 s. Due to the turbulence that occurs in the interaction between the phases, a k-ε viscosity model was defined to analyze the problem. A no-slip and thermal effect condition are applied to all walls.

4. RESULTS AND DISCUSSION

From the simulations performed, it was possible to obtain the temperature gradients, variation of the vapor fraction in the vessel, as well as the average variation of the mass fraction of water inside the straw.

4.1 Validation of the númerical model

To validate the numerical model, an experimental study was carried out, evaluating the variation in water temperature when the straw is immersed in liquid nitrogen. The results obtained experimentally were compared to the numerical results, as shown in Fig. 4.

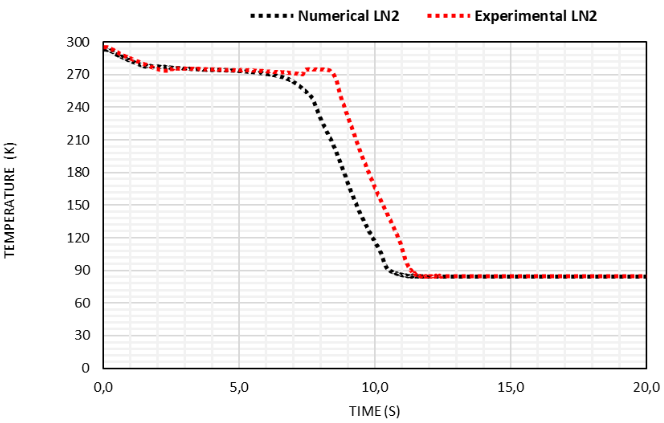

Figure 4. Comparison of the numerical and experimental water temperature curve obtained for validation of the numerical model

4.2 Analysis of nitrogen vaporization in the freezing chamber

Figures 4 and 5 show the formation and change in vapor fraction over time for the liquid nitrogen and slush nitrogen. According to Figures 4 and 5, in both simulated cases (LN2 and SN2) it is possible to verify the formation of vapor at the initial moments and during the process. However, in the case of liquid nitrogen, the formation of vapor is more expressive compared to slush nitrogen. This phenomenon can be related to two factors. The first factor is associated with the initial temperature of the process (77 K, case 1 – LN2 simulation) which is equal to the nitrogen saturation temperature at the considered pressure (101,321 kPa). In this way, the occurrence of steam in the initial moments is faster as any increase in temperature will cause the liquid nitrogen to change phase. In turn, in the case of nitrogen slush (case 2 – SN2 simulation) the initial temperature is 10 K below the nitrogen saturation temperature. The second factor is associated with the density difference between the substances.

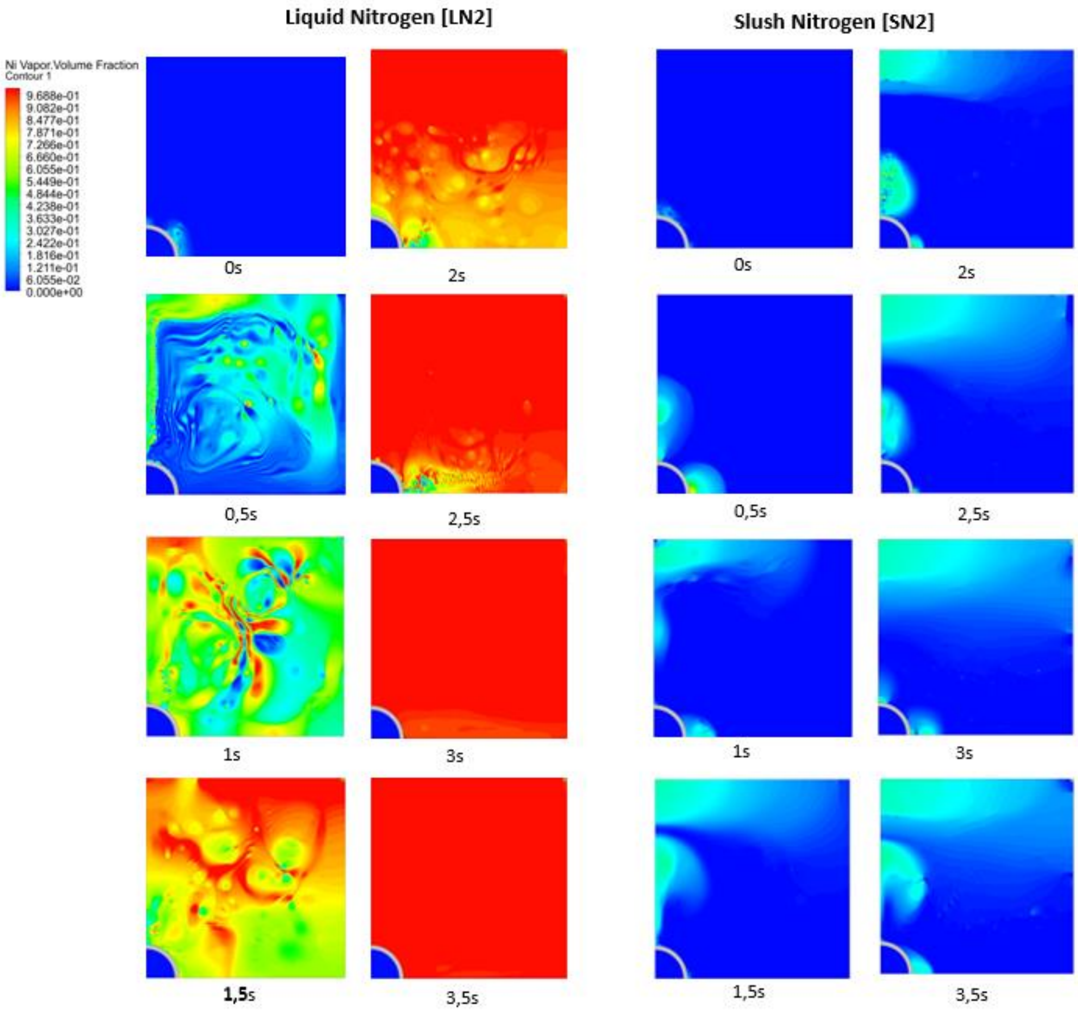

Figure 4. Contours of vapor formation inside the freezing chamber for NL2 (left) and SN2 (right).

The result shown in Figure 4 agrees with reports in the literature. Echlin (2011) concluded that, improved heat transfer occurs because there is less film boiling when a sample is immersed in slush nitrogen. As heat is first transferred to solidified nitrogen, first occurs to melting rather than produce vaporization. Yoon *et al* (2007) in your experimental with oocytes observed that no vapor formation occurred when the sample was immersed in slush nitrogen.

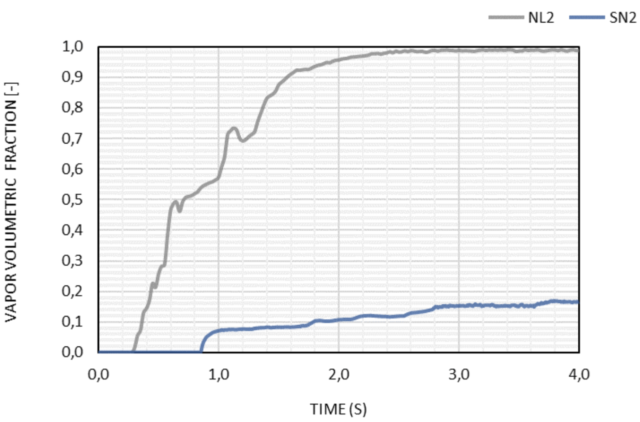

Figure 5. Variation of the nitrogen vapor fraction inside the freezing chamber.

However, according to Figure 5, there is a slight vaporization of the slush nitrogen inside the cooling vessel. This occurrence may be associated with high initial temperature of the external surface of the straw (293 K). Therefore, a pre-cooling of the biological material before diving is recommended in order to reduce the temperature difference between the nitrogen slush and the straw.

4.3 Analysis of water solidification

From the simulations, it was possible to obtain the cooling curve of water and its solidification behavior for each case (LN2 and SN2), as shows Figure 6 and 7, from time step of 3,5 s.

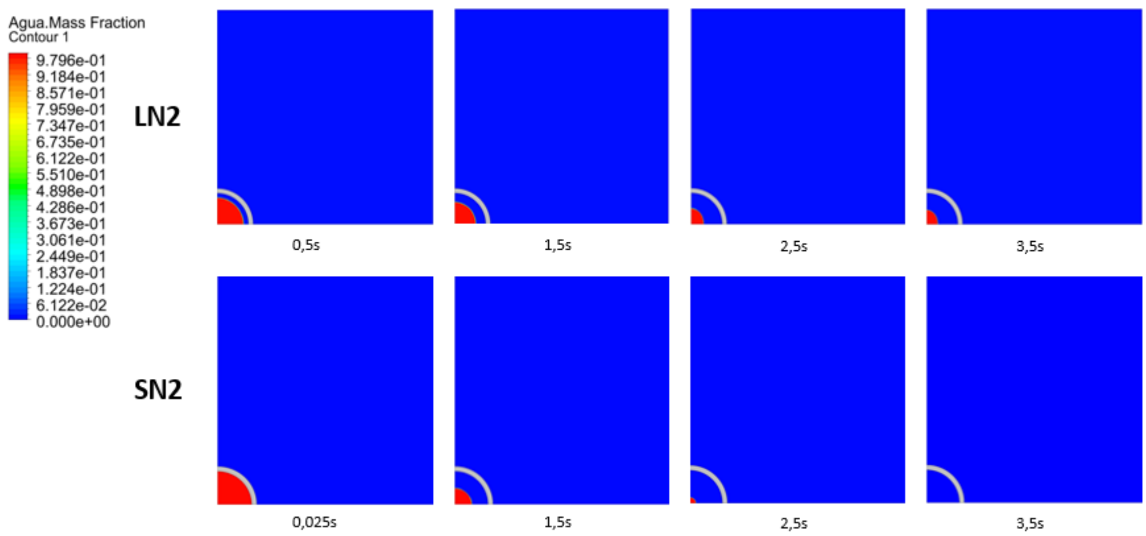

Figure 6. Contours of liquid mass fraction for liquid nitrogen (LN2) and slush nitrogen (SN2).

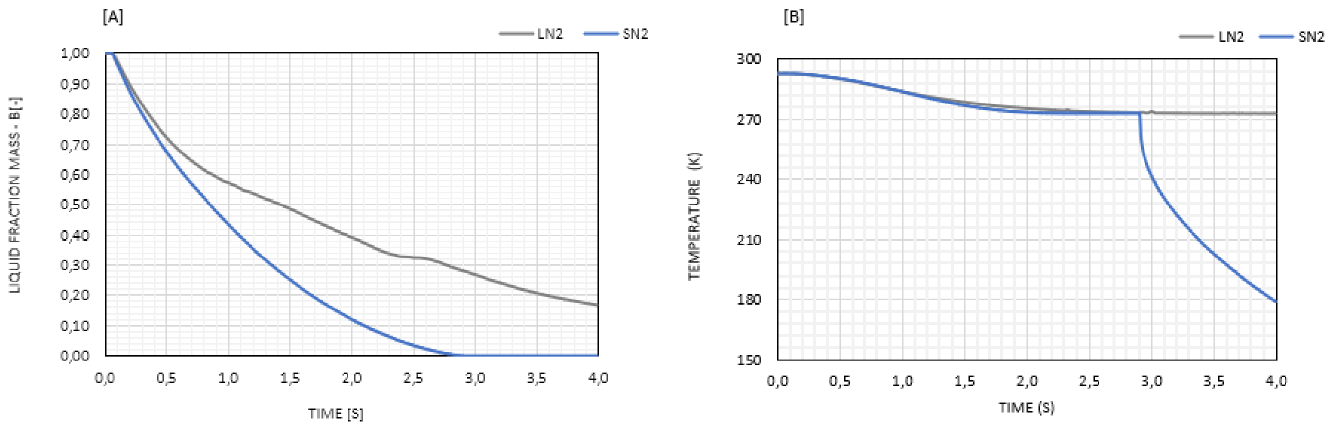

Figure 7. Contours of liquid mass fraction for liquid nitrogen (LN2) and slush nitrogen (SN2).

Due to the low evaporation presented by the slush nitrogen, observed in Figure 4 and 5, it was expected that the water solidification time would be shorter in relation to liquid nitrogen. In addition to the slush being cooler, the absence of steam around the external surface of the straw provides better heat transfer in the system, as suggested by Echlin (2011).

Based on the results presented in Figures 6 and 7, it can be observed that the final temperature (simulation time) at the middle point of the straw is 273.2 K for liquid nitrogen and 175 K for slush nitrogen. Knowing that, in both situations, the initial temperature is 293 K, the average cooling rate in the case of liquid nitrogen is 298.48 K/min and for the nitrogen slush, it is 1769.91 K/min.

It is important to emphasize that the low cooling rate cannot be exclusively associated with the low performance of liquid nitrogen. Experiments and simulations carried out using polypropylen straw showed heat transfer coefficients between 200 to 2000 W/m^{2}K and the cooling rate ranging between 200 to 1.700 K/min, according to Sansinema et al. (2011).

5. CONCLUSION

The vitrification process of biological materials is a very interesting and complex area, as it involves many variables and physical phenomena, which are often difficult to obtain experimentally. Using numerical simulations with CFD, it was possible to analyze a vitrification system using cooling fluids with different characteristics, obtaining its cooling and solidification curves, as well as vapor formation contours for different instants of time.

According to data available in the literature, the nitrogen slush had a much higher cooling rate compared to liquid nitrogen. Its positive performance can be associated with its initial temperature (67 K) and low steam production, factors that provide a higher heat transfer rate. Liquid nitrogen, in turn, had poor performance, due to the large amount of vapor that forms around the straws. The difference observed in the performance of each fluid was reflected in the cooling rate and solidification time of the water. With a cooling rate above 1600 K/min, the nitrogen slush took just 2.8s to reach the complete solidification of the water. In the case of liquid nitrogen, 4s of simulation were not enough to completely solidify the water, as a result of the low cooling rate, around 300 K/min, thus proving the need to create a vitrification mechanism or techniques that aim to overcome this difficulty.

Following this work, experimental analyzes will be carried out to prove the occurrence or not of steam inside the cooling chamber and the replacement of straw by a more thermally conductive material, since the polypropylene used in this simulation had a direct influence on the results obtained.

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