Temperature Control for a Cryogenic Freezer for Bovine Semen Samples for Assisted Reproduction and Genetic Improvement Purposes.

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Abstract

The project presented consists of a temperature control system, based on a PID (Proportional, Integral, Derivative) controller in a pole placement scheme, for a cryogenic freezer that allows a trajectory monitoring control to carry out the freezing of bovine semen samples according to internationally accepted temperature profiles. and thereby try to increase the survival rate of bovine sperm, which is currently only considered to be 50% by traditional means of freezing this type of sample. The system is based on discrete components that are very easy to acquire and handle to offer an alternative in the control of cryogenic systems and processes, seeking a satisfactory cost-benefit ratio. An important point to highlight is that this control system is designed ad-hoc as the first version for a cryogenic freezer that is also under development by the members of this same project.

Keywords:

Temperature Control, cryogenic system, Preservation of bovine semen, PID control, Instrumentation.

1. INTRODUCTION

This project responds to the need to offer a temperature control system for a cryogenic freezer used for freezing bovine semen samples. This need was expressed to us by the "*Asociación Ganadera Local de Durango A.C.*" (Local Livestock Association of Durango A.C.) since the associates require an option for the development of their cattle herds based on assisted reproduction (artificial insemination) to achieve genetic improvement and obtain greater economic and reproductive performance. In the city of Durango, there is a genetic improvement center of the state government linked to the Association, however, the system available is a large cryogenic system for a large number of semen samples and that itself is neither economical nor attractive. The temperature control system described in this work aims to be a viable alternative for its application in cryogenic freezers for applications such as the one mentioned above, for which the problem to be solved is explained below; In the first place, it was thought to adapt an existing and available system, but in the end, it was decided to design and develop a fairly new cryogenic system whose characteristics include that it can be adjustable to the necessary volume according to the number of straws that an associate or a group of them needs. For this, the temperature control system must also be able to adjust to the variable volume and also be able to follow a temperature profile that goes from room temperature to almost 196°C below zero, which is the temperature of liquid nitrogen.

This control is based on the pole placement technique [3] that allows tracking of the temperature profile as the reference signal. The implementation of the control is done using discrete continuous and digital elements in a PID control scheme with a model of a continuous plant. The block diagram of the system is seen in figure 1

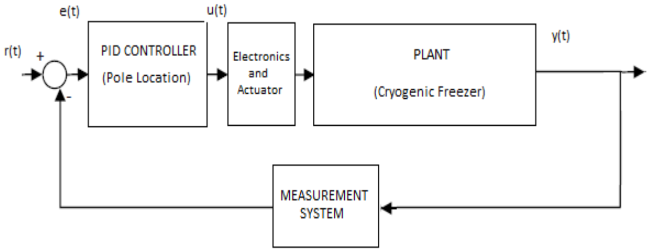


Figure 1. Block Diagram of the Temperature Control System.

2. TEMPERATURE MEASUREMENT

2.1 Cryogenic Temperature Measurement.

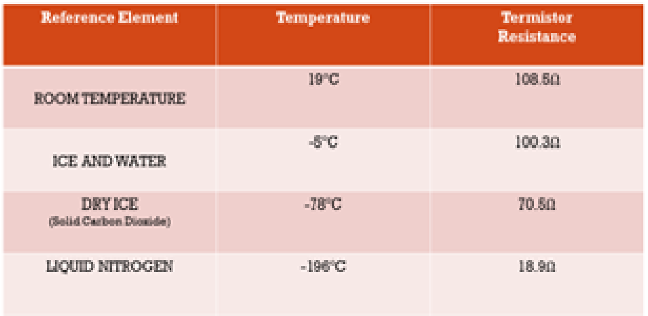
Most general-purpose temperature measurements occur within a fairly limited range between freezing water and boiling water (≈-10°C to ≈120°C), but what happens when temperatures well below 0°C have to be measured? [2]. The laws of physics are still valid, however, the materials undergo important transitions and their characteristics and behaviors change radically. The performance, linearity, and other critical attributes of sensors and their materials change dramatically in very low °C ranges. While familiarity with water turning to ice or steam is common, changes at cryogenic temperatures are much more difficult to grasp and understand. [11].

For temperature reference in this project, we use the cryogenic thermometer "Traceable® model 6458" from Control Company.

2.2 Temperature Measurement in the Control System.

To avoid as much as possible the problems and high costs in materials and sensors for cryogenic temperatures, in this project we decided to prove the temperature sensor Pt100 RTD [12] since it has very linear characteristics in a wide range of temperatures. We practically prove the RTD by testing it to obtain its characteristic curve. The test consisted of obtaining various RTD resistance reference points relative to known temperature values. The values can be seen in table 1.

Table 1. Resistance measurements of RTD Pt100



Reference Element	Temperature	Termistor Resistance
ROOM TEMPERATURE	19°C	108.5Ω
ICE AND WATER	-5°C	100.3Ω
DRY ICE (Solid Carbon Dioxide)	-78°C	70.5Ω
LIQUID NITROGEN	-196°C	18.9Ω

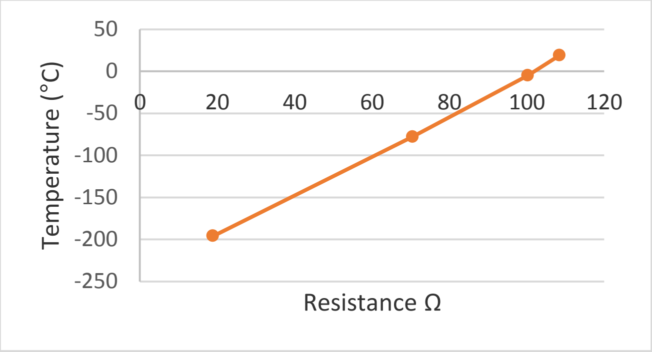


Figure 2. Experimentally obtained characteristic curve of the Pt100 RTD.

As can be seen in figure 2, the RTD has a quasi-linear characteristic in the region of interest for the temperature measurements, which facilitated the design of the meter.

2.2 End actuator handling.

Regarding the final actuator of the control system, which in this case is a 25-watt OMEGA® CYC320-HTR cartridge heater [13], which is placed at the bottom of the container with liquid nitrogen (LN2) in a truncated cone type aluminum sheath to avoid direct contact with the LN2, see figure 3. In figure 4 we can see the cartridge heater in place in the LN2 container. When feeding the heater, it transfers heat to the LN2 which causes the evaporation of nitrogen and with the increase in pressure, it is forced a flow of nitrogen gas whose variation allows temperature control in the cooling chamber of the bovine semen sample freezer.

Figure 3. Cartridge heater and aluminum sheath

Figure 4. Heater in LN2 Container

The heating cartridge power supply is 25 volts of direct current to generate a maximum power of 25 watts, which is enough to vary the pressure of the container with the LN2. To supply the heater, a simple power circuit was designed with a Pulse Width Modulator (PWM) and a MOSFET power transistor. The system diagram for the freezer control final actuator is shown in Figure 5.

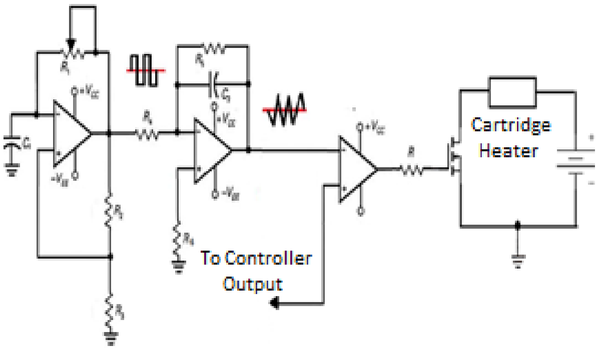


Figure 5. Power circuit for the end actuator.

3. CONTROL SYSTEM DESIGN

3.1 Description of the freezing process of bovine semen samples.

In the specialized literature on the subject, it is mentioned that bovine semen can be preserved by freezing the samples employing nitrogen gas (N2), liquid nitrogen (LN2), or by other means that provide cryogenic temperatures, through programmable automated systems capable of following a certain rate of freezing. [4].

The freezing curves of biological samples of bovine semen are not common in the literature; however, there are references [1] that describe various freezing rates that have been carried out experimentally and have achieved acceptable results. Most of the published works concerning cryopreservation use automated programmable freezing systems at different freezing rates, for which a commonly accepted curve is shown below and is the basis of what is intended to be followed in the project with some small variations in the very low-temperature zone.

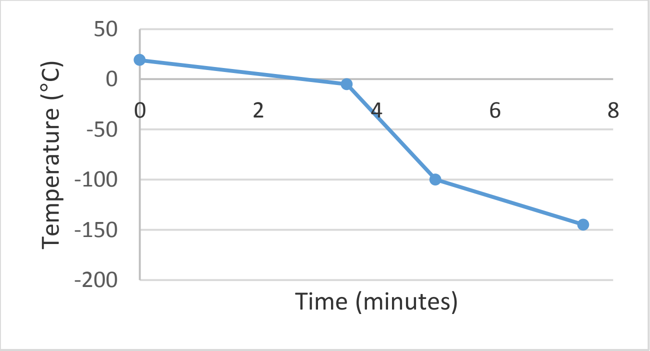
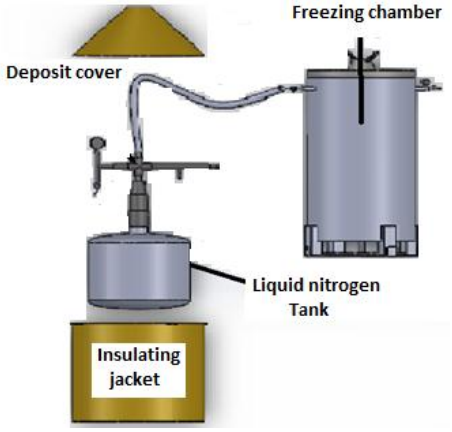


Figure 5. Freezing profile of bovine semen samples [1].

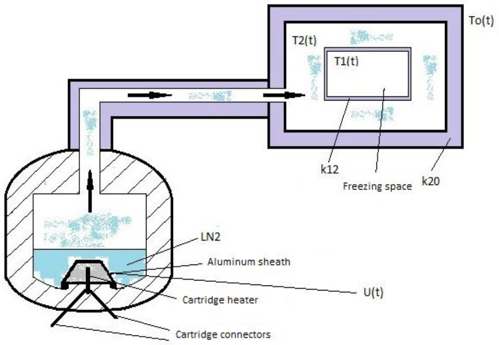
Figure 5 shows the curve with different freezing rates, each one for a specific time, which allows the biological samples to adapt to the ambient conditions by varying the temperature from room temperature to minus 150°C after 8 minutes, the sample can be placed in liquid nitrogen for storage and preservation. The above process offers us the opportunity to obtain a better survival rate of the sample, than just dropping the sample directly into the liquid nitrogen at t=0, which is how it is practically done with a good number of cattle producers in the state of Durango.

3.2 Description of the Plant to be Controlled.

The cryogenic freezer is a thermal system in which the heating element is embedded in the space where the liquid nitrogen is located so that when the temperature of the nitrogen increases, an increment in pressure is generated in the container and the nitrogen gas enters the freezing space where the straws containing the bovine semen samples are located. A schematic diagram of the plant is shown in Figure 6.



a). Drawing of the plant



b). Schematics

Figure 6. Plant to be controlled.

The prototype construction of the cryogenic freezer is shown in figure 7.

Figure 7. Freezer prototype in construction.

The materials with which the plant is built have a great influence on the design and performance of the freezing temperature control system, therefore, to obtain the transfer function, the materials of the thermal system that are considered are glass as straw material, glycerol as bovine semen cryoprotectant, high density expanded polystyrene as insulating material and nitrogen as the transmission medium.

3.3 Transfer Function of the Plant

The plant to be controlled is a thermal system that, due to its structure and construction, the model results in a second-order system, which can be verified with the system of simultaneous equations that are applied to the plant that is written as: [5]

$$m_1C_1\,ST_1(s) = k_{12}T_2(s) - k_{12}T_1(s) \qquad (1)$$

$$\begin{aligned} m_2C_2\,ST_2(s) = k_{12}\big(-T_2(s) + T_1(s)\big) \\ + k_{20}\big(-T_2(s) + T_o(s)\big) + U(s) \end{aligned} \qquad (2)$$

Where:

m_1: Glycerol mass.

C_1: Specific heat of glycerol.

k_{12}: Thermal conductance of glass.

k_{20}: Thermal conductance of expanded polystyrene.

m_2: Nitrogen mass.

C_2: Specific heat of nitrogen.

T_0: Room temperature.

T_2: Medium 2 temperature

T_1: Medium 1 temperature

U: Control signal.

Manipulating the equations with numeric values for the constants and using Cramer's rule, the transfer function for the relationship between T1 and U is obtained:

$$G(s) = \frac{T_1(s)}{U(s)} = \frac{0.0003236}{(s+0.000131034)(s+0.07876)} \qquad (3)$$

Considering $T_0(s)$ as a system perturbation that can be handled.

3.4 Temperature Controller.

The proposed controller for the system is a PID controller designed by pole location whose design generates a control in continuous time. The controller approaches a reference tracker, which, in this case, the reference signal is a succession of ramps with different negative slopes that draw the desired profile for freezing bovine semen samples, see figure 5. The proposed PID controller equation is in its parallel form:

$$PID_{controller} = P + I\frac{1}{s} + D\frac{N}{1+\frac{N}{s}} \qquad (4)$$

Where N is a filter factor for the derivative part.

Due to the characteristics of the reference signal, it is appropriate to think about the pole location scheme for the design of the controller, since this technique is the basis of the design of RST (Reference Signal Tracking) digital controllers [6].

For the design by pole assignment, the plant must be at least of second order and counting on the model of our plant, we proceed to the design of the control taking into account the following design restrictions: Maximum settling time (Ts) of 45 seconds, and a maximum overshoot (Mp) of 5%. The simulation was carried through the *Matlab®* program.

The application of the control in a practical way has not been achieved 100% because the final version of the prototype of the cryogenic freezer is not available yet.

4. DISCUSSION OF THE RESULTS.

With the results obtained from the simulations and some of the partial practical measurements since the final version of the prototype has not been achieved, it can be said that what is obtained is promising in the sense that, concerning the time constants of the controller, the response of the plant is fast enough to follow the temperature profile that is used as the reference of the control system.

Although, the above would only be valid if in the final version of the prototype an efficiency of at least 79% is achieved in the energy transmission from the liquid phase to the gaseous phase of nitrogen, which is the one that comes into direct contact with the straws that contain bovine semen samples.

The graph in figure 8 shows the tracking of the freezing profile of the bovine semen samples and it can be seen that the tracking of the ramps is achieved within 98% and the control corrections to the change in slope are achieved within the first 32 seconds, which is fast enough to ensure a smooth change in freezing temperature and minimally affect the process on the bovine semen in the straws.

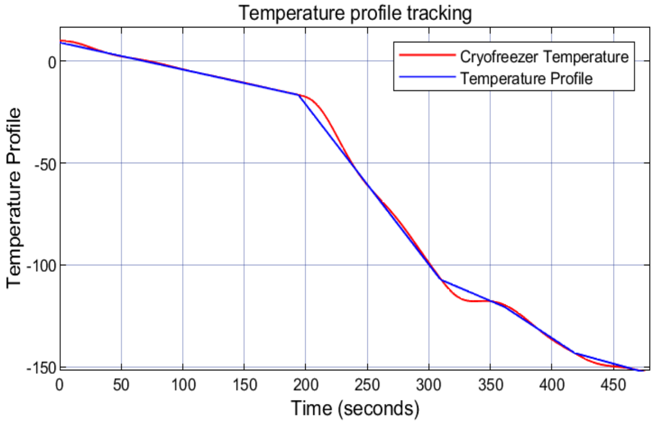


Figure 8. Temperature Profile Tracking

Figure 9 shows the control effort developed by the controller, which does not have sudden changes, and that allows us to ensure that the power circuit that feeds the cartridge heater works efficiently and the necessary power can be transmitted to modify the pressure in the container with the liquid nitrogen.

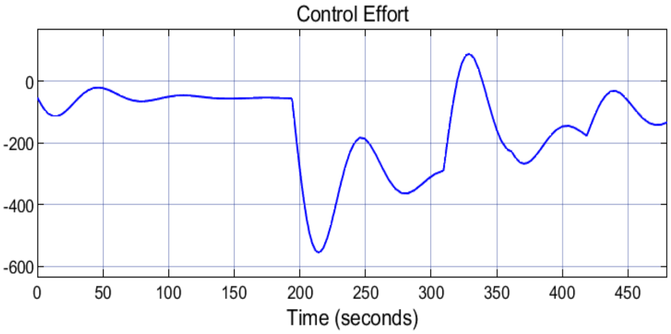


Figure 9. Control signal of the system.

5. CONCLUSIONS

Regarding the results by simulation or practically obtained on the freezer it can be seen that this project is a viable alternative to other cryogenic freezers and applied methods far more expensive for the assisted reproduction of livestock. At the actual stage of the project, it is obvious that is needed to enhance some characteristics of the cryogenic freezer to finally obtain the expected efficiency near the 80% to ensure the results we see in the simulations. At this time, the adaptations to achieve more efficiency are in progress.

6. ACKNOWLEDGEMENT

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7. REFERENCES

[1] Arango, J. E., Durán, C. M., Lizarazo, J. E., & Duarte, J. A. (2017). "*Sistema automatizado para el congelamiento de semen bovino*". Revista Colombiana de Tecnologías de Avanzada.

[2] Bar-Cohen, Yoseph. (2016). "*Low temperature materials and mechanisms".* Taylor & Francis Group.

[3]Castaño, S. A. "*Control PID por Asignación de Polos",* https://controlautomaticoeducacion.com/control-realimentado/control-pid-por-asignacion-de-polos/?unapproved=11306&moderation-hash=3de69dda2d17adef83eb5cb6e9058a00#comment-11306.

[4]Dalal, J., Kumar, A., Dutt, R., Singh, G., & Chandolia, R. (2018). "*Different Cooling Rates for Cryopreservation of Semen in Various Livestock Species: A Review*". International Journal of Current Microbiology and Applied Sciences.

[5] Jacquot, R.G.. *Modern Digital Control Systems .* Routledge, 2nd ed. Marcel Dekker, Inc. (1995).

[6] Landau, I. D., & Zito, G.. *Digital Control Systems (Design, Identification and Implementation),* Springer-Verlag, London, Ltd. (2006).

[7] Ribeiro-Peres, A., Munita-Barbosa, L., Yumi-Kanazawa, M., Mello-Martins, M., & Ferreira de Souza, F. (2014). Criopreservación de espermatozoides bovinos extraídos de la cola del epidídimo utilizando los métodos convencional y automatizado. *Arch Med Vet*.

[8] Santos, M., Sansinena, M., Zaritzky, N., & Chirife, J. (2013). *"How important are internal temperature gradients in french straws during freezing of bovine sperm in nitrogen vapor?",* http://bibliotecadigital.uca.edu.ar/repositorio/investigacion/how-important-are-internal-temperature.pdf

[9] Swartz, J. M., & Lawrence, G. R. (s.f.). Fundamentals for Usage of Cryogenic Temperature Controllers. *Lake Shore Cryotronics Application Note*, 9.

[10] Vishwanath, R., & Shannon, P. (2000). *Storage of bovine semen in liquid and frozen state.* https://www.sciencedirect.com/science/article/abs/pii/S0378432000001536?via%3Dihub i

[11] A. Alekseev, Basics of Low-temperature Refrigeration Linde AG. Munich, Germany.

[12] Pallás, R.A., "Sensores y Acondicionadores de Señal", Alfaomega-Marcombo, 4ª ed. (2008).

[13] https://mx.omega.com/pptst/CYC320.html.