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Redesign, adaptation and control of a thermal cycler module

Rediseño, adaptación y control de un módulo termociclador

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Abstract

This paper presents the study, re-design and adaptation of a thermal cycler. The aim of this work is to increase its performance and capacity without diminishing the durability of the equipment. The Cole Parmer 1095-00 model is used for the study. It is proposed to modify the current temperature selector by a digital one that allows controlling the temperature in a range of 0-100 ° C. In addition, it is proposed to include a controller to maintain the water level at the desired operation point, including the system to add a replenishment stage. New components are included as a screen to show the operating temperature, the start date of the process, the remaining time and an alarm to indicate the end of the cycle. An interface is also developed to monitor and control the operation of the remote system in real time. The monitoring system is developed using the Labview platform®.

Thermal Cycler, Reverse Engineering, Classic Contro

Resumen

En este trabajo se presenta el estudio, re-diseño y adaptación de un termociclador con el objetivo de actualizar su operación, aumentar su capacidad de trabajo, sin disminuir la durabilidad del equipo y mejorar su rendimiento. Para el estudio se utiliza el modelo Cole Parmer 1095-00 y se propone modificar el selector de temperatura acutal por uno digital que permita controlar la temperatura en un rango de 0-100°C. En el sistema, Además, se plantea incluir un controlador para mantener el nivel del agua en el punto de operación deseado, incluyendo al sistema agregar una etapa de reabastecimiento. Se incluyen nuevos componentes como una pantalla para mostrar la temperatura de operación, la fecha de inicio del proceso, el tiempo restante y una alarma para indicar el término del ciclo. También se propone el desarrollo de una interfaz para monitorear y control en tiempo real la operación del sistema a distancia a través de un programa ejecutable y otro fijo, ambos desarrollados en la plataforma de Labview®.

Termociclador, Ingeniería Inversa, Control Clásico

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Introduction

Polymerase chain reactions (PCR) are simple techniques widely used in the field of molecular biology to amplify and detect sequences of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), especially to perform serological tests, incubation procedures, agitation, activation, biomedical, pharmaceutical, among many others (Tamay de Dios, et al., 2013). A thermal cycler or thermal sequencer is used to carry out the tests of the chain reactions, whose purpose is to duplicate DNA fragments to obtain multiple copies in an automated way, controlling the chain reaction efficiently and quickly, both for qualitative amplifications and for quantitative (Armas, 2006).

According to Cortazar et al. (2004), the first thermocyclers that were used worked with mechanisms that moved a tube rack between several thermostatic baths at pre-established times. However, modern termocyclers use metal blocks to control temperature, especifically the heating-cooling process, offering the possibility of programming temperature and time. Decisive factors in the quality of the amplification are the stability of the temperatures and the rapidity in the passage from one temperature to another. It is important to note that this type of process from the point of view of control are considered slow or slow response systems.

In current thermocyclers available on the market, heating and cooling occurs thanks to the cells or the Peltier effect, which consists of heating or cooling a junction between two different metals (isothermal interface) when passing current through it. When the current is reversed, the flow direction of the heat is reversed (Sandoval, 2012). The cost of thermal cyclers can vary greatly depending on the number of functions they have. In general, for the purposes of this work, thermocyclers are grouped into analog and digital, as shown in the figure 1. The main characteristics of both equipment are the agitation system that prints the heating medium a carefully controlled movement to keep the temperature as uniform as possible, the temperature selector and the choice of time or duration of the process (MTAS, 2004). Unlike analogs, digitals offer other functions such as liquid level control and system monitoring (Reyes et al., 2018).

In thermocyclers, water is usually used as a liquid medium, but it also allows working with oil. The temperature ranges in which the thermal cyclers are operated normally oscillate between the ambient temperature and 60 ° C. Table 1 shows the temperature range and the means used for operation in commercial thermal cyclers.

(a)

(b)

Figure 1 Thermocycler (a) analog and (b) digital available in the market

Class	Rank
Low temperature	25-60°C
	25-100°C
High temperature	25-275°C

Table 1 Operating ranges of commercial thermal cyclers

When it is required to operate at temperatures above 100 °C, it is essential to use fluids other than water because its boiling point is 100 °C. So for higher temperatures oils are used (whose boiling points are much higher).

Currently the Faculty of Marine Sciences of the Ucol has the thermocycler model Cole Parmer 1095-00, this device is used for the cultivation of bacteria, micro algae, fungi, phytoplankton and other organisms. This model has basic functions for temperature control, however, it is desired to improve its performance at low cost. At this time, there are cultivation processes that must be monitored by a certain perso to verify that the process has not been interrupted (causing loss of data), so it is necessary to review the process constantly.

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Another disadvantage is that in the current model there is evaporation of the liquid (water), so it is necessary to monitor the process and add water to maintain the desired level. So thinking about improving the performance of the currently equipment was born the proposal of this work that is to automate and maximize the operating functions of the thermal cycler. To reach the objective, it is proposed to modify the temperature selector that is performed manually in the current model and modify it by a digital selector that allows to control the temperature in a range of 0-100 ° C.

In addition, it is proposed to include a controller to maintain the water level at the desired operating point automatically, for which a replenishment system must be added and new components are included, such as a display to show the operating temperature, the start date of the process, the remaining time and an alarm to indicate the end of the cycle. It also proposes the development of an interface to monitor and control in real time the operation of the remote system through an executable program and a local one, both developed in the Labview® platform.

Design and description of the system

For demonstration purposes, figure 2 shows the figure of the Cole Parmer 1095-00 thermal cycler.

Figure 2 *Cole Parmer 1095-00 Thermal Cycler*

In general, the design of thermocyclers can be divided into two areas. An stage that includes the control algorithm, the energy regulation and the measurement system (for sensors).

The other stage involves the design of the thermomechanical system: the sample tray (the metal alloy that makes up the block), the sensors, the cooling module or Peltier cell, the insulation of the tray, the protection stages and the cooling system for the electronic boards. Figure 3 shows the proposed block diagram. The main block includes the framework for the thermal cycler and the interface for reading and processing data.

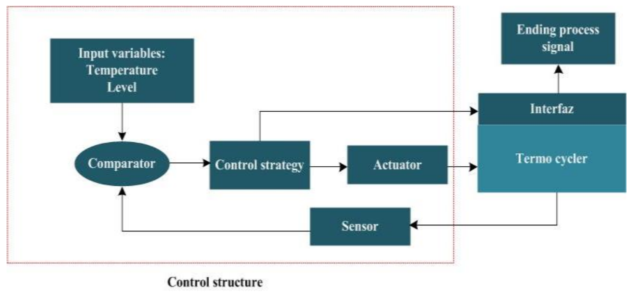
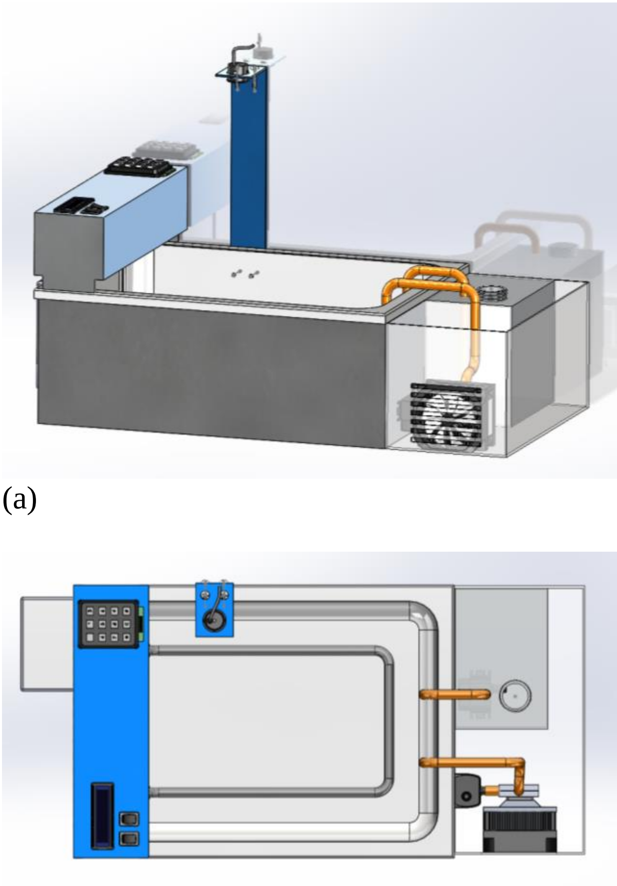


Figure 3 Generalized block diagram of the main components of the thermal cycler

Figure 4 shows the proposed design including the improvements described. The schematic was developed in Solidworks®. Above figure 4(a) se muestra una vista dimétrica del prototipo. Figure 4 (b) the trimetric perspective of the prototype. By means of the numerical matrix (keyboard) that is observed in figure 4 (b), it will be possible to enter the values for the temperature, the level of the desired liquid (important to maintain the crops) and the time of the process; these data can be displayed on the LCD screen. In addition, the system has a temperature sensor that feeds back the current values to the control algorithm, and this data is updated on the indicator screen.

In the proposed system, the user can place the time that the process will last in hours and minutes. The system allows a maximum time of 96 hours to be set. On the other hand, figure 5 shows in greater detail the cooling system and the refueling tank that is proposed to compensate for the evaporation of the liquid during the process in the thermal cycler.



(a)

(b)

Figure 4 Modifications proposed the Cole Parmer1095-00 thermocycler, (a) dimmetric view of the structure, (b) aerial view

To circulate the water was included a submersible pump that has interchangeable nozzles for rigid or flexible hoses and an oxygenated hose.

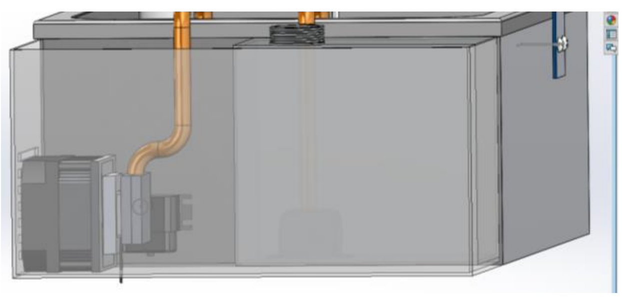


Figure 5 Refueling tank and cooling system

The pump is sealed to prevent water from permeating the electrical and electronic system. It also has a flow regulator of four levels and a regulating knob with a capacity of 280 liters per hour. Peltier cells were used for the cooling system.

Figure 6 shows the electronic components that will be responsible for carrying out the proposed tasks in the thermal cycler.

Two Arduino® systems are used to achieve the proposed objetives. The Arduino® Mega is responsible for detection, level control, time control and user interface (keyboard and display). On the other hand, the Arduino® UNO is responsible for temperature control, activation of water pumps, circulation system and activation of Peltier cells. It is important to note that the output of the temperature sensor sends information to the two Arduino® cards and that these two communicate through the serial port. Then, in the following sections, each of these stages is described.

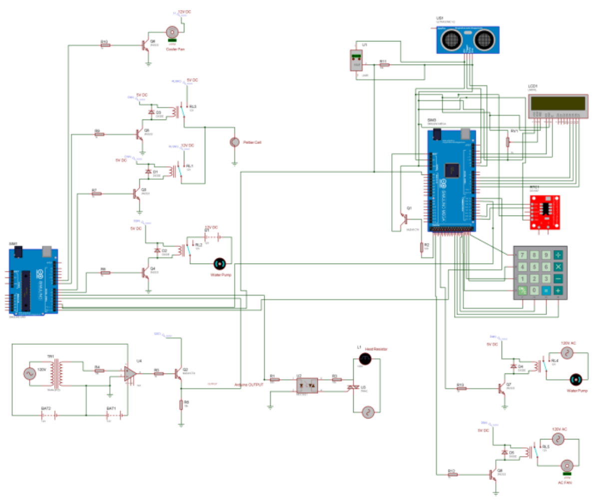


Figure 6 General electronic diagram proposed the thermal cycler

Temperature control

To monitor the temperature, the YSI 44018 sensor is used, which has an accuracy of ±0.15º C (it guarantees that the DNA samples to be replicated do not degenerate). In addition, a conditioning stage was included to linearize the thermistor output, as well as an INA125 instrumentation amplifier to condition the output range (0-5V) and to couple the digital signals. Figure 7 shows the circuit used for the simulations. According to the current specifications of the thermal cycler, it is required to control the temperature in the range of 0 to 100 ° C allowing steps of ±0.5 ° C.

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As for the control stage, this will be responsible for generating the necessary operating conditions to maintain the desired value at the point of operation knowing the temperature value, the desired operating time and the water level. In this way, the control algorithm sends a signal to the power stage so that it is responsible for increasing or decreasing the temperature in the system.

On the other hand, the power stage will be responsible for regulating the temperature and the cooling system. For the above, TRIACs of type BTA 24800 with operating ranges that support up to 25 A and 800 V are used. These devices are used to control the firing of the thermal resistances (operating at 12 A) and to enable the cooling system using pumps to send the hot water Peltier cells that help in the cooling process (Ayllón, 2012).

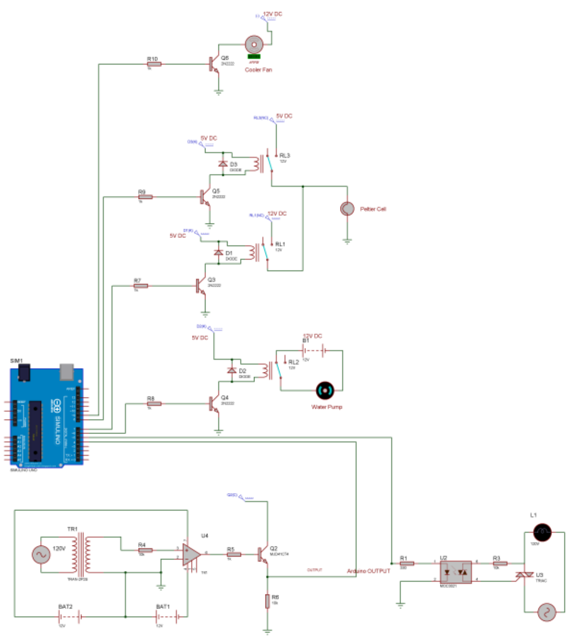

Figure 7 Electronic circuit proposed for temperature control

In this proposal, the Peltier cell is activated by means of 2 relays that receive the information sent by the power stage through an active IGBT switch; such that a 5V signal is fed back when the temperature error is within a range of 1-10 ° C and 12 Volts when the error is greater than 10 ° C. Within the modifications, a ventilation system was fitted to help the cooling of the electronic components. It should be noted that these monitored signals are sent for processing to the Arduino® UNO.

Level control and process time

To monitor the variable level in the thermal cycler, the ultrasonic sensor JSN-SR04T was used. This sensor is able to resist humidity and high temperatures. On the other hand, the Maxim / Dallas DS1307 integrated circuit, which is compatible with the Arduino® cards, is used to record the date and the real-time count of the duration of the process. Among the features of this integrated circuit it can be mentioned that the date is automatically adjusted at the end of the month for months with less than 31 days and it even includes corrections for leap year. Figure 8 shows the proposed electronic circuit for level, time and interface control. It can be seen that the Arduino® Mega will carry 2 AC actuators, such as the re-supply pump and the thermocycler's propeller that allows the temperature to be spread uniformly in the water (García et al., 2018). Both will be activated by means of relays.

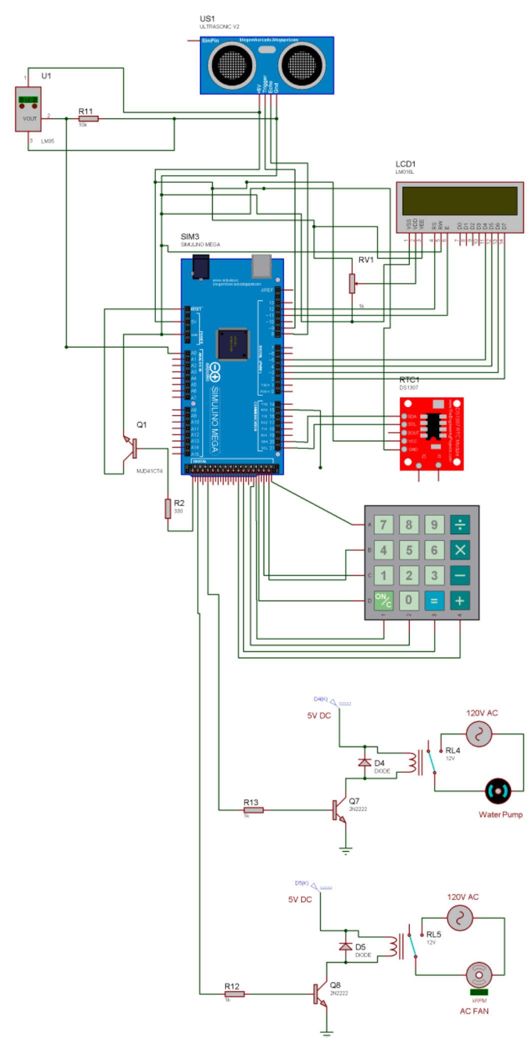

Figure 8 Proposed electronic circuit for level, time and interface control

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Implementation of the thermal cycler

Below are the different modifications that were made to the thermal cycler.

Figure 9 shows the adaptation of the system for entering and displaying data, at the beginning of each process the user is asked to enter the desired temperature values, the duration of the process (time) and the desired water level. Once the data has been entered, the user can view the values entered through the LCD screen. The keys allow the following actions: A = enter data, B = delete data, C = restart the system, D = pause, # = "enter".

Figure 9 Keyboard and display of the thermal cycler

As can be seen in figure 10, on the side is located the start button and the communication port that links the thermal cycler with the control interface in Labview®.

Figure 10 Communication port and on / off button

The cooling system is achieved with the arrangement of a pump resistant to high temperatures that drives the hot water to an aluminum coil that is coupled to the Peltier cell, as shown in figure 11. By circulating the hot water through the aluminum coil, the temperature drops quickly and is fed back into the main vat.

Hydraulic hose resistant to high temperatures was used for the connections of the cooling system to give greater durability to the equipment and a purge was placed to empty the water from the main tub if necessary, in a fast and safe way.

The control of the Peltier cell is of the ON / OFF type and manages 2 intensities, depending on the percentage of error the adequate intensity will be given to the cooling system. For the above, 5 states were assigned that help determine the level of the liquid, each level has a difference of 1.5 cm. The circuit contains 8 leds which will work as level indicators (1cm per level). Through the interface, the user will be able to enter the desired level in the thermocycler and level control system will allow to maintain that current level throughout the cycle, with a duration of time given by the user.

Figure 11 Regulation and cooling system for water in the thermal cycler

Control interface in Labview®

As mentioned in the introduction, within the proposed improvements to the thermal cycler, it is proposed to monitor the thermocycler process in real time and with the ease of doing it remotely (if necessary); to achieve the above, an executable interface was made using Labview® software that can be run on any computer equipment. The program allows to evaluate the status of the temperature and the remaining time of the cycle in the thermal cycler.

Figure 12 shows the block diagram that is used to generate the graphic interface in Labview®. Because the variable to control is the temperature and its response is slow, it was decided to implement a controller of the proportional type, capable of regulating the output at the desired value.

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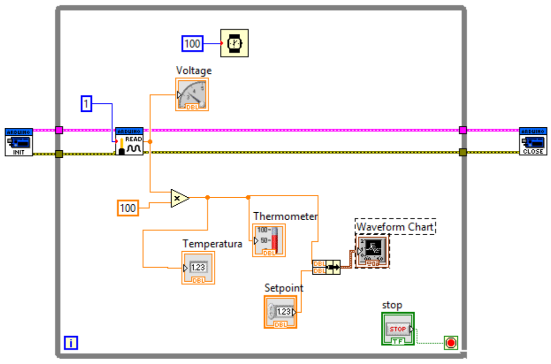

Figure 12 Block diagram in Labview®

Figure 13 shows a screen print of the main operation window generated in Labview®. In the upper left part, the time set for the process is shown and in the lower part, the desired value at which the temperature is to be regulated, both graphically and numerically. For demonstration purposes, the experimental results are shown for a process in which the temperature was set at 45 ° C and the time interval was set at 5 hrs. It is important to mention that according to the laboratory specifications, the executable program is used only for monitoring purposes. However, the program is also installed in a central computer that establishes a link between Labiew® and Arduino® and that allows making changes into the system, such as modifying the operating point of the thermal cycler or the time.

Experimental tests were carried out with different temperatures and at different levels. The stabilization time was set at 200 ms; this respone is considered adequate for this type of systems, classifed as slow. Figure 14 shows the results for a test in which the temperature was modified from 35 to 45 ° C with a liquid level of 40% of the total capacity, in the graphic it can be observed how the variable changes to reach the desired value shown in figure 13.

Figure 13 Proposed electronic circuit for level, time and interface control

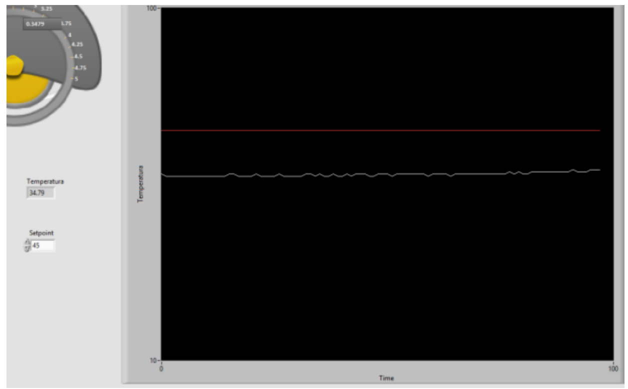

Figure 14 Transient temperature from 35 to 45 ° C

Conclusions

In this work, a proposal to improve the performance of a commercial thermal cycler for the model Cole Parmer 1095-00 was presented. Among the improvements made to the system, it can be highlight the modification of the temperature selector for a digital one that allows introduce the desired temperatures in the range of 0-100 ° C with variations of ± 0.5 ° C and that includes a controller to maintain and regulate the level of the temperature liquid used in the process through a replenishment system. In addition, an interface was developed to monitor the operation of the remote system in real time through an executable program developed in Labview®, which allows control actions to be exercised from the central system in Labview®. The developed system is low cost and allows to continue using an old model.

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