ISSN No:-2456-2165

Development of an Automatic and Real Time pH Monitoring and Adjustment System to Optimize Antigen Production in Bioreactors

Andrianirina S. T.[1], Razafindrafara M. S.[1,3], Ramahefarison T. O.[1], Solofoarimana T.[1], Maminiaina O. F.[1, 2, 3]
[1]Malagasy Institute of Veterinary Vaccines, Antananarivo, Madagascar
[2]Department of Veterinary Science and Medicine, Faculty of Medicine, Antananarivo Madagascar
[3]Department of Zootechnical, Veterinary and Fish Research, National Center for Applied Research in Rural Development, Antananarivo, Madagascar

Abstract:- Bioreactors, also called fermenters, are used in the production of vaccine antigens. However, various parameters (temperature, pressure, agitation, pH, oxygen concentration) must be carefully controlled to ensure the right conditions inside the bioreactor. Indeed, the good control of these parameters ensures a successful production and a high yield. In this work, an automatic real-time pH monitoring and adjustment system is developed. The system is composed of a device made with various materials and specific computer programs. The device is needed to detect the pH value in the bioreactors while the programs are used to control and parameterize the device. The device is called "pH control kit" and consists of a pH sensor, a processing unit, a switching hardware and a transmission hardware. The device is controlled by software and two types of programs called "pH control", "Arduino" program and "Java" program respectively. The "pH control kit" works for different types of bioreactors, however, the control programs can be customized for different types of antigens. The results show that it is possible to automatically detect and adjust the pH value in a culture medium in real time. Thus, the device and control programs can be a valuable tool for optimizing bioreactor-based antigen production processes.

Keywords:- *bioreactors, pH, automatic, pH control kit, control programs, antigens.*

I. INTRODUCTION

Since the days of Jenner and Pasteur through the induction of an immune response to infectious diseases, vaccination has become a widely applied intervention [1]. Indeed, vaccination represents the best strategy for preventing infectious diseases. Moreover, the manufacture of vaccines is crucial for the control and eradication of diseases in the world [2]. Vaccine manufacturing is composed of several fundamental steps such as isolation, purification, bacterial multiplication and vaccine formulation [3]. In addition, bioreactors are processes used in the manufacture of vaccine antigens [3]. The use of bioreactors has advantages due to the existence of many options to adjust the process parameters to improve antigen production and to reduce manufacturing time. [2]. In addition, bioreactors provide optimal conditions for multiplication of vaccine strains to optimize the yield of bacterial biomass. However, several factors must be considered for the proper operation of a bioreactor, such as the type of process (batch, continuous, etc.), control of temperature, pH and oxygen supply, sterilization of materials and equipment used, and maintenance of the environment to ensure that it is free of contaminating microorganisms [4,5].

In the production of bacterial vaccines, pathogens are grown in bioreactors using a developed medium [6]. Using bioreactors, optimal conditions of process parameters such as pH value must be monitored and if possible controlled to ensure high bacterial yield. This can be achieved by developing new monitoring methods and exploring alternative uses of existing techniques that can provide additional process information and ensure consistent product quality [7]. Since bacteria grow in a specific pH [8], the formation of organic acids and bases (lactic acid, acetic acid, biogenic amines, etc.) as a result of metabolic activity in microorganisms can vary the pH of the culture medium. [9,10]. On the other hand, changes in pH have a significant impact on cell viability and growth rate [11,12]. To obtain an ideal environment for bacteria, real-time adjustment of pH by addition of acid or base solution is essential. An automation system maintains the required quality and cost consistency of production without any manual intervention. [13]. Nowadays, automation of analytical systems has been used in many fields, such as clinical, pharmaceutical and biomedical applications [14]. The automatic system has played an important role in qualitative and quantitative analysis [5]. The objective of this work is thus to design a system for automatic monitoring and adjustment of pH in real time.

II. METHODOLOGY

A. System components

The bioreactor essentially consists of a reactor vessel and various materials such as peristaltic pumps, containers containing NaOH and HCl, conduction tubing, a pH sensor (electrode) and a computer (Figure 1). However, additional materials and computer programs are designed in this study.

a) pH electrode and its amplification module

In this study, a pH meter kit with a combined, glass type electrode and an amplifier with an analog output (Figure 2) is used to measure pH. The specification of the kit is summarized in Table 1.

ISSN No:-2456-2165

The signal coming from the electrode is processed by operational amplifiers (CA3140AMZ, TL081BCDG4) with a TC1121COA voltage regulator to have an analog output (Figure 3)

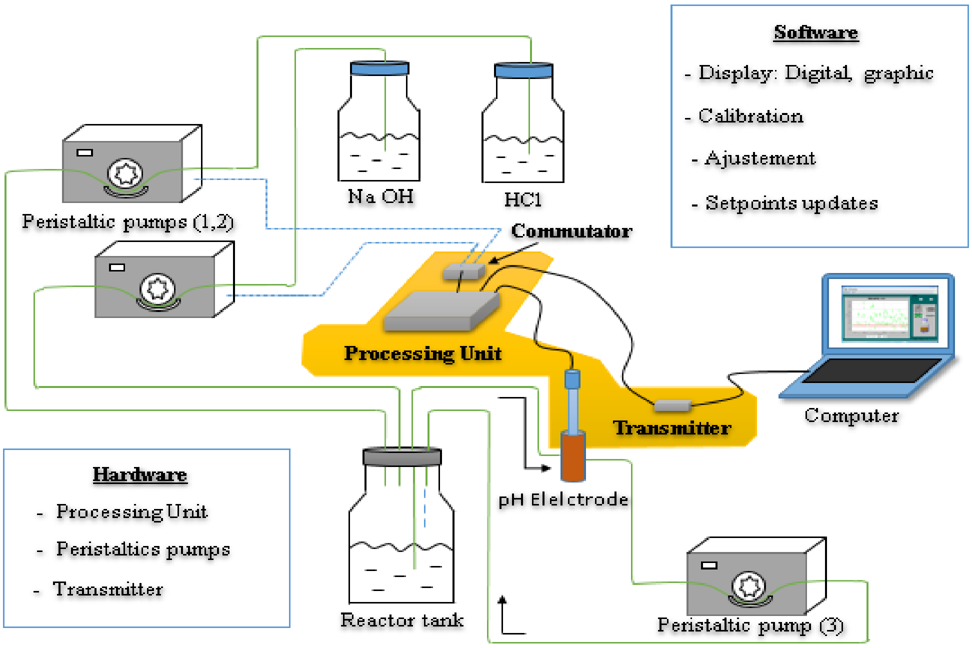

Fig. 1: Synoptic diagram of the system

Fig. 1: Combination electrode; Electrode and pH amplification module (DFRobot)

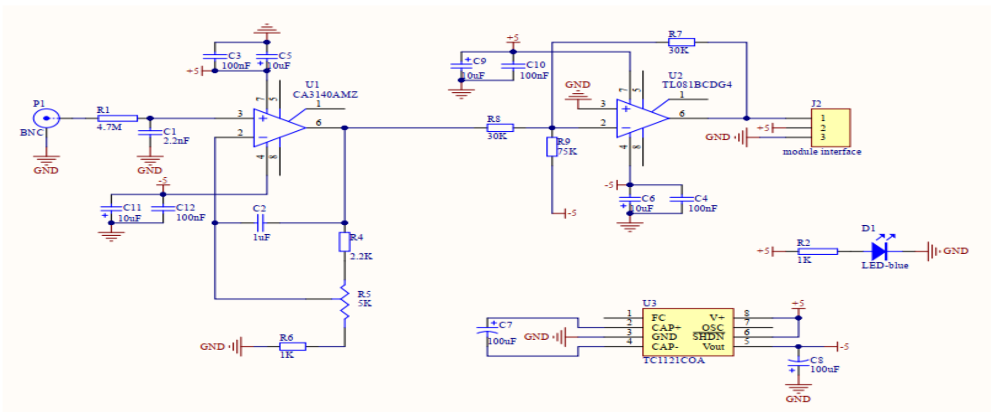

Fig. 2: Internal structure of the pH amplifier module (DFRobot)

Designation	**Values**
Module power :	5,00 V
Module size	43 x 32 mm (1.69 x 1.26")
Measuring range:	0 - 14PH
Measuring temperature	0 - 60 ℃
Accuracy	± 0.1pH (25 ℃)
Response time	≤ 1min
Connector Sensor	BNC
pH2.0 interface (3 foot patch)	
Gain adjustment potentiometer Power indicator	

Table 1: Specification of a pH meter kit

B. *Design of materials*

a) Processing unit

A processing unit consists of an Arduino board, a Serial MAX-485 amplifier and a relay. An Arduino board with Atmel AVR architecture (ATMega 328p) is used (Figure 4). The specification of the ATMega 328P is shown in Table 2. The processing unit plays various roles: it receives the analog value from the pH electrode amplifier; it converts the value to digital format and compares this value with the setpoints (pH min and pH max). It triggers or switches on peristaltic pumps according to the results of the comparison and updates the values obtained. It also calibrates the pH meter and can adjust the pH value to the standard.

The Serial MAX-485 amplifier (Figure 5) is used to connect the control computer and the measurement device. The MAX485 chip that establishes a wired transmission is used. It operates with a 5V supply and a current of 300 µA [15]. The module provides four 10K pull-up resistors on the data lines: two 20K resistors (R5, R6) on the A/B differential lines and a 120 ohm resistor (R7) that passes between the output differential lines (OUT-MAX).

The relay is a simple electronic circuit and consists of an electromagnetic switch (Figure 6). It is created with amplification by an NPN transistor and short circuit protection as a diode. It works when the electric current flows through the system [16].

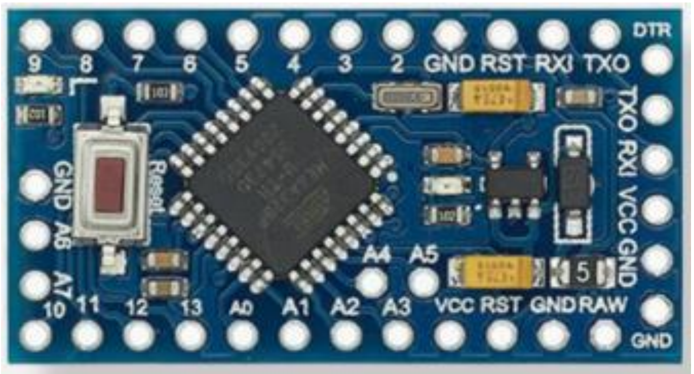

Fig. 3: Arduino pro mini

Designation	Value
a processor	8-bit RISC architecture
Flash memory for programs,	32 KB
SRAM memory () for data,	2 KB
EEPROM memory for data	2 KB
all the clock logic;	16 MHz
Timers/ Counters (T/C)	8 and 16 bits
- generation of PWM signals, - serial communication interfaces (UART, SPI, TWI compatible I2C...), - Analog to digital converter (A/D converter)	

Table 2: ATMega 328P specification

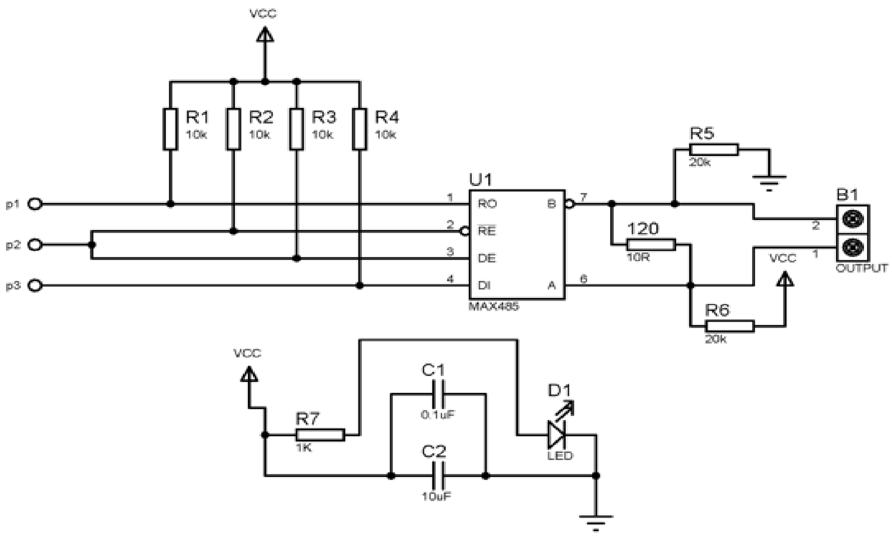

Fig. 4: Diagram of a RS-MAX 485 module

Fig. 5: Relay 5 V 10 A (DEVBOT)

b) Switching equipment
The switching hardware is required to activate or deactivate the control of the two peristaltic pumps (Figure 7). It is separate from the processing unit and powered by another source to avoid disturbance of the measurements.

c) Amplification material
The amplification equipment is designed to increase the link between the equipment and the computer (Figure 8). The longer the distance between these two tools, the more likely it is that the signal will be lost or destroyed.

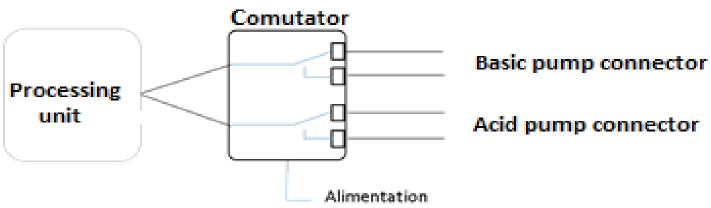

Fig. 7: Synoptic diagram of the peristaltic pump switch

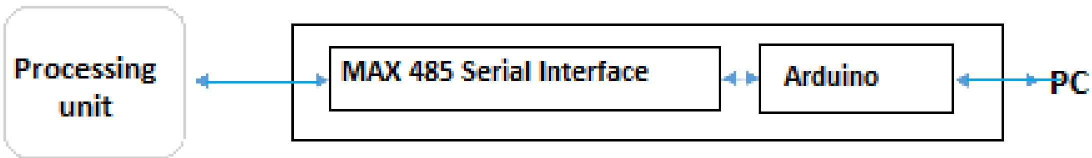

Fig. 8: Block diagram of the transmitter connected to the computer

C. Computer program design

The Arduino board acquires the analog values from the pH amplifier module, controls the relays according to these values, executes the commands and stores the values from the computer program (Figure 9). The computer programs are designed to process the data from the pH sensor and to adjust in real time the pH required for a culture (Figure 10).

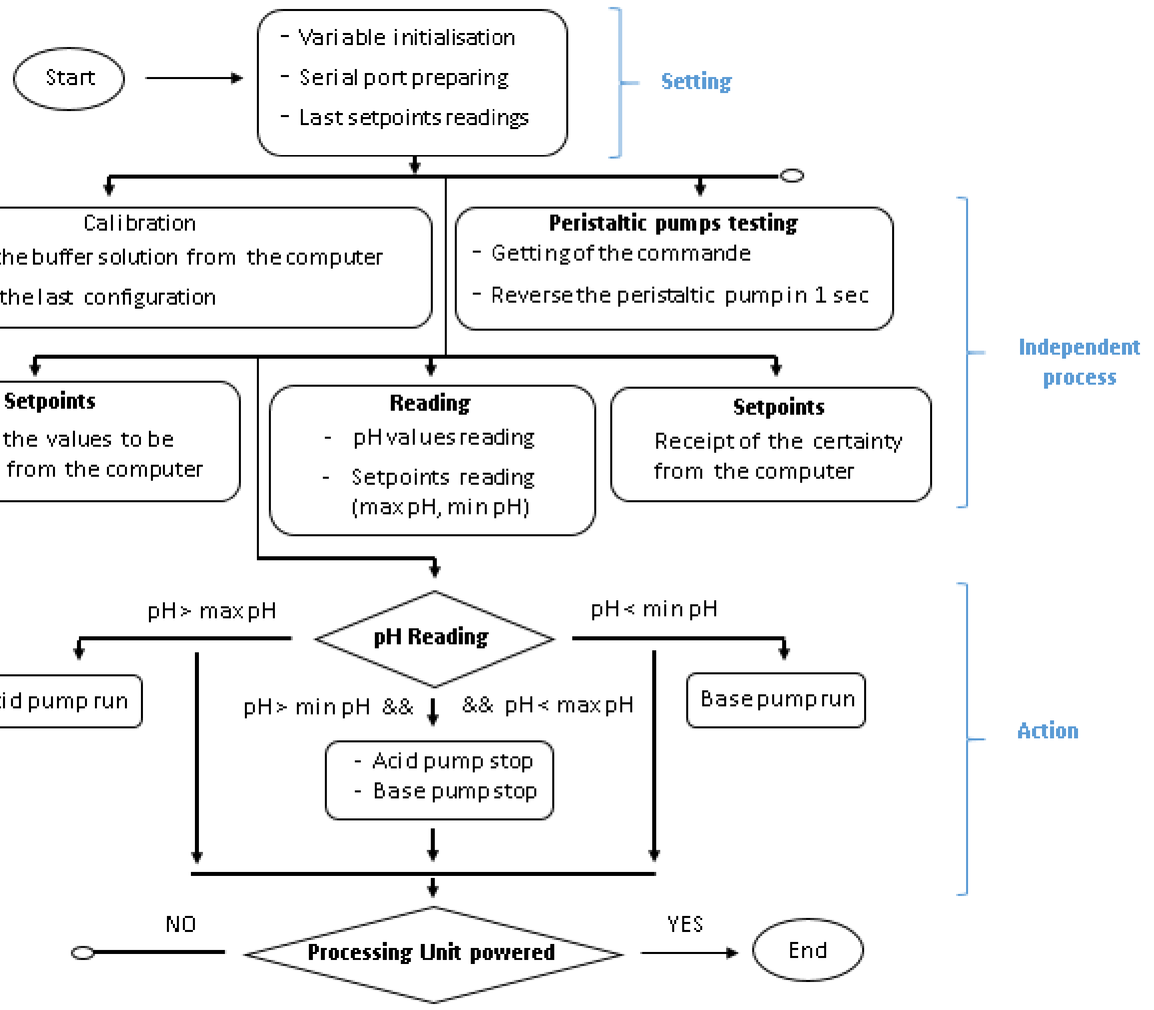

Fig. 6: Flowchart of the program running on the Arduino

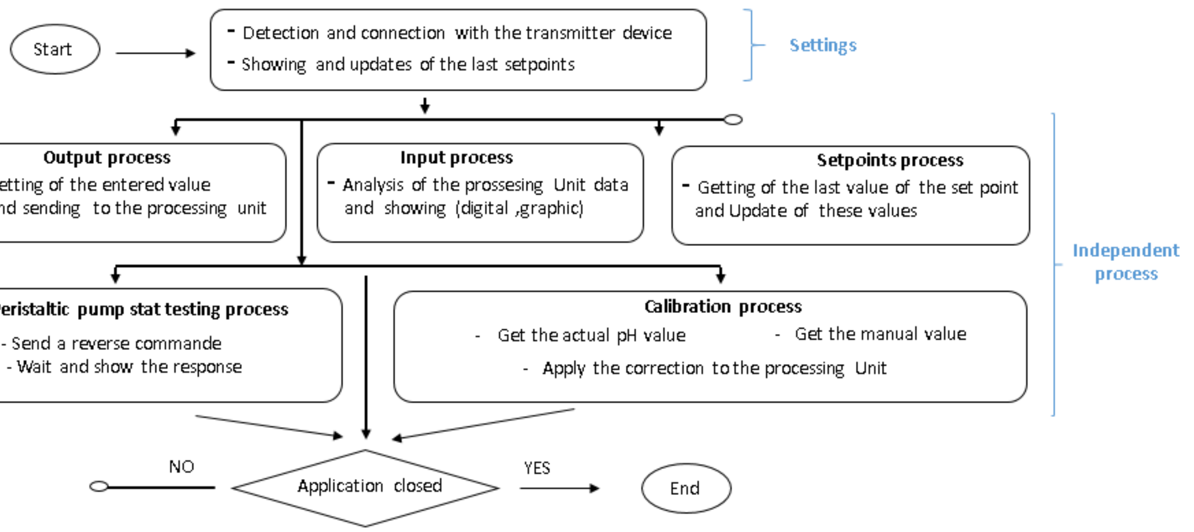

Fig. 7: Organization chart of the computer program

III. RESULTS AND DISCUSSIONS

A. *Materials designed*

a) General presentation of the prototype
The prototype designed for real-time pH acquisition and adjustment is shown in Figure 11. The prototype is called "pH-Control-kit" formed by the pH electrode (Figure 11A), processing unit (Figure 11B), switching unit (Figure 11C) and amplification unit (Figure 11D). The design of this prototype is ideal for real-time monitoring of pH. Indeed, the absence of continuous information on parameters such as pH limits the efficiency of a bioreactor [17].

b) Processing unit
The box containing the processing unit (L=12cm; l=8cm and h=3cm) is shown in Figure 12. It is composed of a pH, an Arduino board, a transmitter, a power supply and a relay.

c) Switching equipment
The box that contains the switchgear is shown in Figure 13 (L= 6cm; l= 4cm and h= 3cm).

d) Amplification equipment
The amplification equipment is found in a box with a dimension L= 7cm; l= 3.5cm and h = 3cm (Figure 14). Faced with the distance of the processing unit and the control computer, we had to design a signal amplifier to communicate these two pieces of equipment. It is equipped with an Arduino board, a MAX-485 transmitter and a LED for data indication. This equipment is connected to the computer with a USB cable type A-micro.

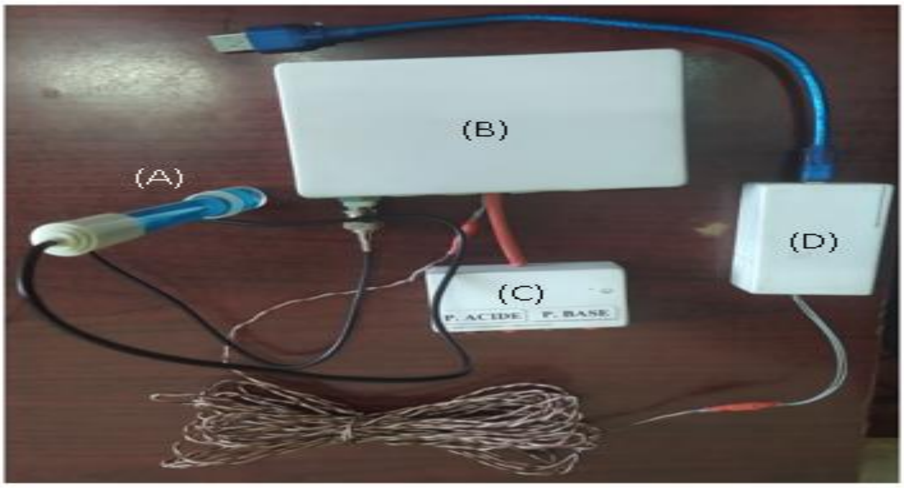

Fig. 8: Overall view of a continuous or on-line pH acquisition system

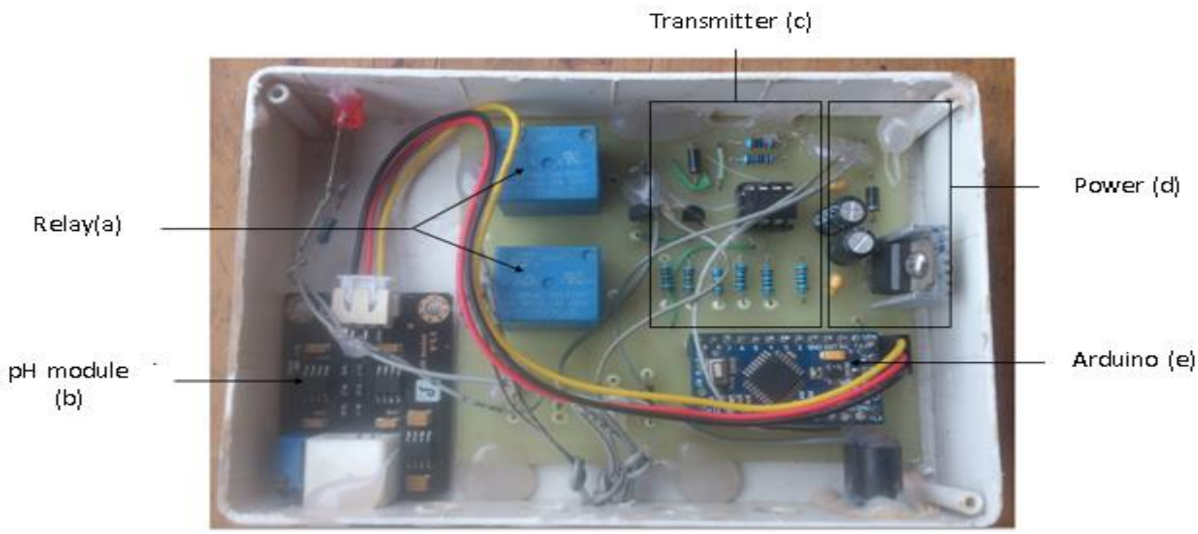

Fig. 9: Internal view of the processing unit

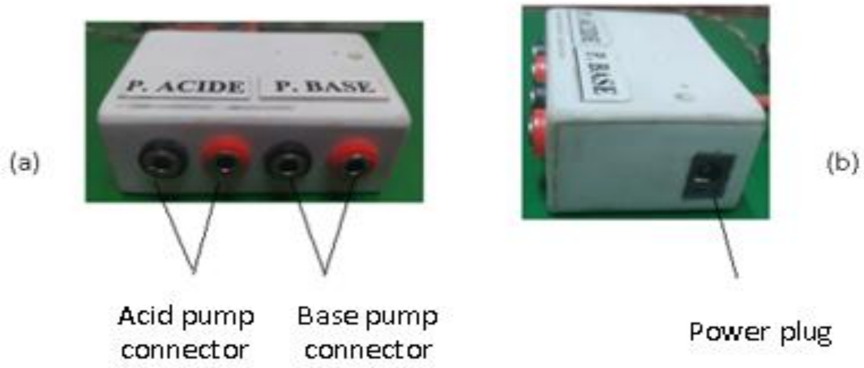

Fig. 10: Switching equipment

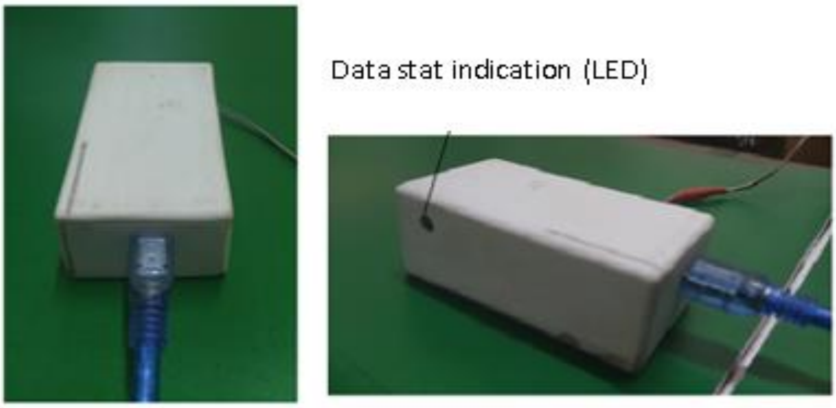

Fig. 11: Signal amplification equipment

B. Programs and software developed

a) pH Control software

To control the system, a software program called "pH Control" was developed. It performs tasks on the processing unit and has four parts: the configuration area, the set point area, the chronological display area and the graphic display area (Figure 15). The configuration area has three menus: the first on the left concerns the creation or opening of a measurement. The second menu in the middle is used to manage access to the interface, connection to the amplifier device, calibration of the hardware, adjustment of the values, updating of the set point, testing of the peristaltic pumps and display of the values. The last menu contains the user guide and the program description.

Fig. 15: Visual representation of the "pH control" software version 1.0

The software interface displays three tabs: Measurement, Settings, Help (Figure 16). In the "Measurement" menu there are three menus for creating a new measurement, opening an existing measurement and a tab for quitting the application. In the "Parameter" menu there are seven windows with tasks such as accessing the interface, connecting the software to the instrument, calibrating the instrument, adjusting the pH values obtained during a measurement, configuring the minimum and maximum pH values, checking the status of the peristaltic pumps and the interface display mode.

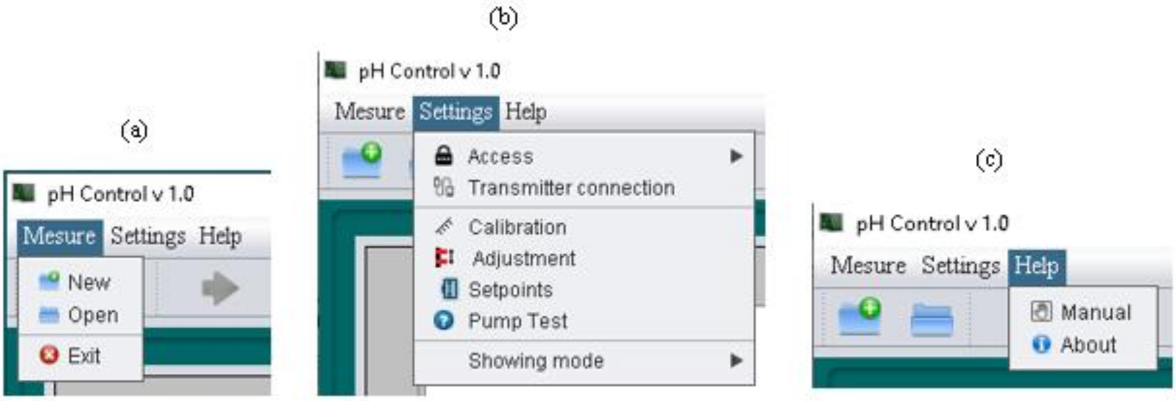

Fig. 16: pH control

b) Arduino program
The Arduino program controls the peristaltic pumps via the relay. It also ensures communication with the "pH control" software. In the program, the minimum and maximum pH value to be respected is declared and initialized after each measurement. If these values are not met, the peristaltic pumps are triggered. This is why functions have been created for updating the data (Setup block) and sending the data to the software (Loop block), as shown in Figure 16.

```
double actual_pH_Value;
double min_cons = 6.85;
double max_cons = 7.12;
double last_min_cons = 6.80;
double last_max_cons = 7.20;
String get_conf, setconf;
const int acid_pump = 4;
const int base_pump = 5;
void setup() {
      Serial.begin(9600);
      pinMode(acid_pump,OUTPUT);
      pinMode(base_pump,OUTPUT);
      update_conf();
}
```

Setup bloc

```
if (actual_pH < min_cons) {
  digitalWrite(base_pump, LOW);
} else if (actual_pH > max_cons) {
  digitalWrite(acid_pump, LOW);
} else {
  digitalWrite(acid_pump, LOW);
  digitalWrite(base_pump, LOW);
}
send_data();
```

Loop bloc

Fig. 17: Arduino startup parameters instruction

c) Java program
A Java programming language is also developed for hardware tracking and configuration. Generic code portions, usable by several applications, are created. All elements are created as objects and then separated by several classes. Figure 17 shows the extracts of the program blocks.

C. pH electrode

Potentiometric measurement of pH value is performed using a pH electrode that works with a reference electrode. The two parts are often constructed as a single sensor called a combination electrode. At its output appears a voltage that depends on the concentration of hydrogen ions H+ or, more strictly, hydronium ions H3 O+ [18]. The electrode delivers a signal that is proportional to the activity of H+ ions within a solution according to Nernst's law [19]. Detection of the pH of the medium can be achieved using a variety of sensors: electrolyte-filled sensors based on porous glass electrodes, MOSFET-based ISFET pH sensors, pH sensors based on optical properties, potentiometric sensors [20]. However, the glass pH electrode is still widely used because of their excellent repeatability, long life and accurate Nernstian response [21]. On the other hand, the glass pH electrode are fragile and require regular maintenance in terms of electrolyte renewal and present fouling problems when used in complex media [20].

ISSN No:-2456-2165

D. Arduino board

The Arduino microcontroller is a computer on a single integrated circuit that includes a processor, RAM, some form of ROM, and I/O ports [22]. It contains a processor core, memory and input/output equipment. Thus, it has the general structure of common computers, but with lower performance [23]. The Arduino microcontroller is a very popular board and is known for its superiority. It is flexible, compatible, user-friendly and has many libraries for different creativities [24]. It is also a tool that can be used to develop programs for free use and is cheaper than their similar components. In this study, the Arduino board is connected to the computer through the MAX-485 IC. Currently, wireless transmission via Wifi, Bluetooth, radio is still the most used because of the simplicity of installation. However, it often causes transmission errors due to noise. Therefore, the use of wired transmission via MAX-485 IC is adopted in this study. In addition, this type of transmission has strong anti-interference capability and can be used in very noisy environments, such as industrial automation.

E. User's manual of the pH control software

With this system, we have written a user's manual in order to help the users to master all the manipulations as well as to solve some problems that may occur. By following this guide, the user will know the physical indications of the materials as well as their installation. He will also know the step-by-step use of the equipment and the system. The presentation of the manual is shown in figure 18a (software version) and figure 18b (paper version).

```
String min_pH = "";
String max_pH = "";
String path = Working_path.absoluteFilePath + "\\Configure\\CF.txt";
String loaded_data = "";
try {
    loaded_data = "";
    Working_path.get();
    FileInputStream inputStream = new FileInputStream(path);
    InputStreamReader reader = new InputStreamReader(inputStream, "UTF-8");
    int character;
    while ((character = reader.read()) != -1) {
        loaded_data += ((char) character);
    }
    loaded_data = "";
    if (loaded_data.length() != 0) {
        for (int i = 0; i < loaded_data.length(); i++) {
            if (loaded_data.charAt(i) == '.') {
                min_pH = "";
                int a = i;
                while (loaded_data.charAt(a) != '/') {
                    min_pH += loaded_data.charAt(a);
                    a++;
                }
                int b = a + 1;
                while (loaded_data.charAt(b) != ',') {
                    max_pH += loaded_data.charAt(b);
                    b++;
                }
                min_pH_txt.setText(min_pH);
                max_pH_txt.setText(max_pH);
            }
        }
    }
} catch (Exception e) {
    System.out.println("Setpoints update error");
}
```

Fig. 18: Interface connection program

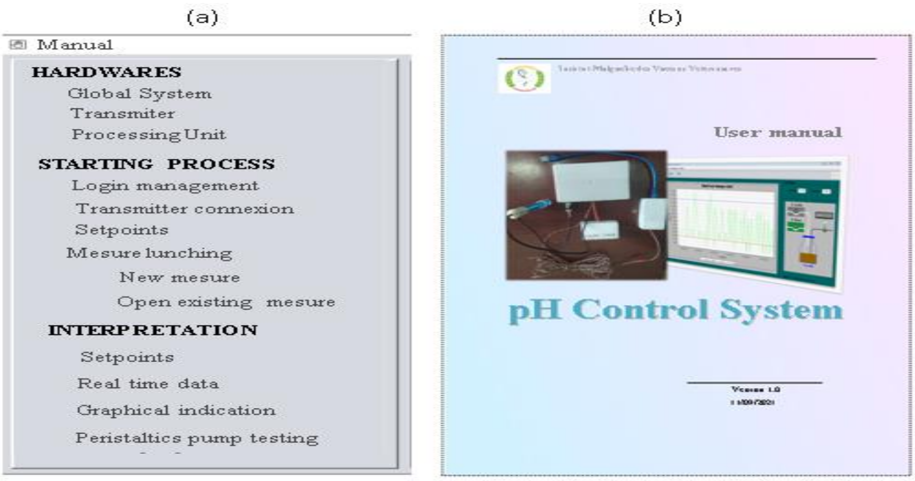

Fig. 19: pH control software user manual

IV. CONCLUSION

Automatic and real-time monitoring of parameters involved in antigen production is essential. In the present study, a new system for automatic detection and determination of pH in real time was presented by designing suitable materials and programs. The hardware consists of pH electrode, processing, switching and amplification unit. In parallel, programs and software used on computer are developed: program "Arduino", program "java" and software "pH control". With the prototypes and the designed programs, satisfactory results were obtained. These results proved to be promising for the detection and automatic adjustment of the pH value. However, developing automatic and real-time control systems does not necessarily mean developing a foolproof system for all eventualities. Thus, improvements in efficiency and robustness should be made from time to time for the economic viability of the product. In the future, we will install systems to detect and automatically adjust other parameters such as optical density, oxygen level, nitrogen level and temperature.

REFERENCES

[1.] Bozzola E, Bozzola M, Calcaterra V, Barberi S, Villani A (2013) Infectious diseases and vaccination strategies: how to protect the "unprotectable"? ISRN preventive medicine 2013: 765354-765354.
[2.] Gallo–Ramírez LE, Nikolay A, Genzel Y, Reichl U (2015) Bioreactor concepts for cell culture-based viral vaccine production. Expert Review of Vaccines 14: 1181-1195.
[3.] Jozala AF, Geraldes DC, Tundisi LL, Feitosa VdA, Breyer CA, et al. (2016) Biopharmaceuticals from microorganisms: from production to purification. Brazilian journal of microbiology : [publication of the Brazilian Society for Microbiology] 47 Suppl 1: 51-63.
[4.] Schmidell W, de Almeida Lima U, Borzani W, Aquarone E (2001) Biotecnologia industrial-vol. 2: Engenharia bioquímica: Editora Blucher.
[5.] Gaydhane MK, Mahanta U, Sharma CS, Khandelwal M, Ramakrishna S (2018) Cultured meat: state of the art and future. Biomanufacturing Reviews 3: 1.
[6.] Gomez PL, Robinson JM (2018) Vaccine Manufacturing. Plotkin's Vaccines: 51-60.e51.
[7.] Randek J (2020) Advancement of sensor technology for monitoring and control of upstream bioprocesses: Linköping University Electronic Press.
[8.] Madigan MT (2003) Anoxygenic phototrophic bacteria from extreme environments. Photosynthesis research 76: 157-171.
[9.] Adamberg K, Kask S, Laht T-M, Paalme T (2003) The effect of temperature and pH on the growth of lactic acid bacteria: a pH-auxostat study. International Journal of Food Microbiology 85: 171-183.
[10.] Arena ME, Manca de Nadra M (2001) Biogenic amine production by Lactobacillus. Journal of Applied Microbiology 90: 158-162.
[11.] Yoon SK, Choi SL, Song JY, Lee GM (2005) Effect of culture pH on erythropoietin production by Chinese hamster ovary cells grown in suspension at 32.5 and 37.0 degrees C. Biotechnol Bioeng 89: 345-356.
[12.] Basu S, Bose C, Ojha N, Das N, Das J, et al. (2015) Evolution of bacterial and fungal growth media. Bioinformation 11: 182-184.
[13.] Liljequist V (2017) Development of a Bioreactor Simulator for supporting automation software test and verification.
[14.] Elpa DP, Prabhu GRD, Wu S-P, Tay KS, Urban PL (2020) Automation of mass spectrometric detection of analytes and related workflows: A review. Talanta 208: 120304.
[15.] Parikh K, Dave H, Kareliya N, Kumar B, Raval V, et al. (2017) Monitoring AC Drive by using RS485 & GSM Module.
[16.] Tjandi Y, Kasim S (2019) Electric Control Equipment Based on Arduino Relay. Journal of Physics: Conference Series 1244: 012028.
[17.] Chimmiri V (2004) Advances in Monitoring and State Estimation of Bioreactors. Journal of scientific and industrial research 63: 491-498.
[18.] Wiora A, W.J., 2018 Nov 23. Laboratory Tests of pH Electrodes in Terms of Industrial Applications Checking Stabilities of Their Parameters and Their Influence on Uncertainties of Measurements. Sensors (Basel
[19.] Stebel K., Choinski D. (2015) Performance improvement for quasi periodical disturbances in ph control. Advances in Electrical and Computer Engineering 15 (1): 123-134 - URL: http://dx.doi.org/110.4316/AECE.2015.01017.
[20.] Mitra S, Murthy GS (2022) Bioreactor control systems in the biopharmaceutical industry: a critical perspective. Systems Microbiology and Biomanufacturing 2: 91-112.
[21.] Clarke DJ, Blake-Coleman BC, Calder MR, Carr RJG, Moody SC (1984) Sensors for bioreactor monitoring and control - a perspective. Journal of Biotechnology 1: 135-158.
[22.] Hussain A, Hammad M, Hafeez K, Zainab T (2016) Programming a Microcontroller. Int J Comput Appl 155: 21-26.
[23.] Schubert TW, D'Ausilio A, Canto R (2013) Using Arduino microcontroller boards to measure response latencies. Behavior Research Methods 45: 1332-1346.
[24.] Olayinka AA, Emmanuel AF (2021) Distance Measurement and Energy Conservation Using Arduino Nano and Ultrasonic Sensor. American Journal of Electrical and Computer Engineering 5, 40-44.