Universidad Autónoma de San Luis Potosí

Instituto de Física
(Physics Institute)

Master's Thesis:

THERMAL GRADIENTS GENERATION BY GRAPHENE INK DEPOSITION:
EVALUATION OF BACTERIA DISTRIBUTION

Thesis Advisors:

Dr. Mildred Quintana Ruíz
Dr. Vanesa Olivares Illana

Author:

Daniel A. González Aradillas

Now days graphene has become very important and its properties are very interesting due to a wide range of possible applications. In this work we studied graphene inks that rise their temperature when an electrical current is applied to them, and we have built a system to study bacteria behavior on temperature gradients generated by graphene inks deposition. Bacteria responds to changing thermal environment by moving towards or away from a particular location, in particular we studied E. Coli response according to temperature gradient generated by graphene inks to observe the effect of temperature over bacteria.

"You never change things by fighting the existing reality. To change something, build a new model that makes the existing model obsolete"

R. Buckminster Fuller

Acknowledgements

I want to thank Dr. Mildred Quintana Ruiz and Dr. Vanesa Olivares Illana for guiding me along this journey of discovery and for pushing me to achieve more than I expected, for helping me to understand confusing phenomena occurred on results, and for being patient with my ways and my style of work that is not very orthodox. I am very thankful.

Table of Contents

1. Introduction

1.1. Carbon Allotropes

Carbon has several allotropes, or different forms in which it exists. Interestingly, carbon allotropes span a wide range of physical properties: diamond is the hardest naturally occurring substance, and graphite is one of the softest known substances. Diamond is transparent, the ultimate abrasive, and can be an electrical insulator and thermal conductor. Conversely, graphite is opaque, a very good lubricant, a good conductor of electricity, and a thermal insulator. Allotropes of carbon are not limited to diamond and graphite, but also include buckyballs (fullerenes), amorphous carbon, glassy carbon, carbon nanofoam, nanotubes, and others.

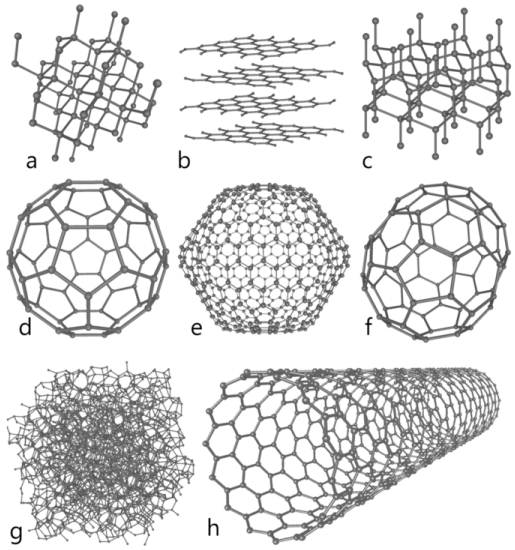


Fig. 1 - Some allotropes of carbon: a) Diamond, b) Graphene sheets, c) Lonsdaleite, d-f) Fullerenes (C_{60}, C_{540}, C_{70}); g) Amorphous carbon, h) Single walled carbon nanotube.

1.1.1 Graphene

Graphene is a semi-metal with small overlap between the valence and the conduction bands (zero bandgap material). It is an allotrope (form) of carbon consisting of a single layer of carbon atoms arranged in a hexagonal lattice. It is the basic structural element of many other allotropes of carbon, such as graphite, diamond, charcoal, carbon nanotubes and fullerenes .[1]

1.2. What an Ink is?

"An ink is a liquid or paste that contains pigments or dyes and is used to color a surface to produce an image, text, or design. Ink is used for drawing or writing with a pen, brush, or quill. Thicker inks, in paste form, are used extensively in letterpress and lithographic printing" (https://en.wikipedia.org/wiki/Ink).

"Ink can be a complex medium, composed of solvents, pigments, dyes, resins, lubricants, solubilizers, surfactants, particulate matter, fluorescents, and other materials". The components of inks serve many purposes; the ink's carrier, colorants, and other additives affect the flow and thickness of the ink and its dry appearance. (https://en.wikipedia.org/wiki/Ink).

Ink formulas vary, but commonly involve two components:

- Colorants
- Vehicles (binders)

Inks generally fall into four classes: [2]

- Aqueous
- Liquid
- Paste
- Powder

Colorants

Pigment inks are used more frequently than dyes because they are more color-fast, but they are also more expensive, less consistent in color, and have less of a color range than dyes.[2]

Pigments

Pigments are solid, opaque particles suspended in ink to provide color.[2] Pigment molecules typically link together in crystalline structures that are 0.1–2 μm in size and comprise 5–30 percent of the ink volume.[2] Qualities such as hue, saturation, and lightness vary depending on the source and type of pigment.

Dyes

Dye-based inks are generally much stronger than pigment-based inks and can produce much more color of a given density per unit of mass. However, because dyes are dissolved in the liquid phase, they have a tendency to soak into paper, making the ink less efficient and potentially allowing the ink to bleed at the edges of an image[2].

To circumvent this problem, dye-based inks are made with solvents that dry rapidly or are used with quick-drying methods of printing, such as blowing hot air on the fresh print. Other methods include harder paper sizing and more specialized paper coatings. The latter is particularly suited to inks used in non-industrial settings (which must conform to tighter toxicity and emission controls), such as inkjet printer inks. Another technique involves coating the paper with a charged coating. If the dye has the opposite charge, it is attracted to and retained by this coating, while the solvent soaks into the paper. Cellulose, the wood-derived material most paper is made of, is naturally charged, and so a compound that complexes with both the dye and the paper's surface aids retention at the surface. Such a compound is commonly used in ink-jet printing inks[2].

An additional advantage of dye-based ink systems is that the dye molecules can interact with other ink ingredients, potentially allowing greater benefit as compared to pigmented inks from optical brighteners and color-enhancing agents designed to increase the intensity and appearance of dyes.

A more recent development in dye-based inks are dyes that react with cellulose to permanently color the paper. Such inks are not affected by water, alcohol, and other solvents. As such, their use is recommended to prevent frauds that involve removing signatures, such as check washing. This kind of ink is most commonly found in gel inks and in certain fountain pen inks[2].

1.3. Inks based on Graphene

Printed electronics offer an attractive alternative to conventional technologies by enabling low-cost, large-area, flexible devices that have the potential to enable unique advances in varied applications such as health diagnostics, energy storage, electronic displays, and food security. The main advantages of this technology include digital and additive patterning, reduction in material waste, and compatibility with a variety of substrates with different degrees of mechanical flexibility.Graphene is a prominent contender as a metallic component in printed electronic devices due to its high conductivity, chemical stability, and intrinsic flexibility.[3]

1.4. Temperature gradient

"A temperature gradient is a physical quantity that describes in which direction and at what rate the temperature changes the most rapidly around a particular location. The temperature gradient is a dimensional quantity expressed in units of degrees (on a particular temperature scale) per unit length. The SI unit is Kelvin per meter (K/m)." (https://en.wikipedia.org/wiki/Temperature_gradient)

Other authors have studied thermal gradients effect on bacteria growth, such as Nithya Murugesan et. al[4]. In that work, they created a thermal gradient by using two water flows on the endings of a culture cell to induce heat in one ending and to extract heat on the other ending. That gives as result two different temperatures on the edges and a thermal gradient along the cell (fig. 2).

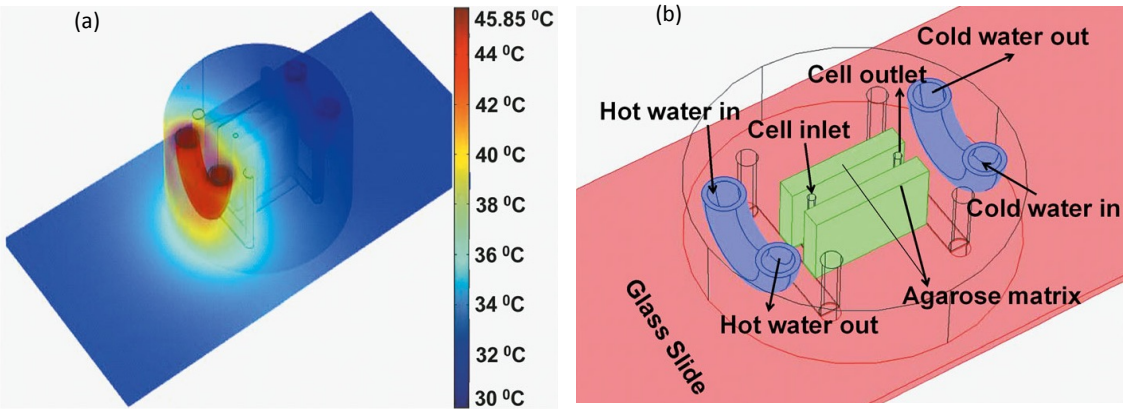


Fig. 2 - Device design and simulation. (a) COMSOL generated schematic view of the device for thermal gradient generation. (b) COMSOL generated view of the microfluidic device simulated for thermal gradient generation. [12] (Image taken from Effect of gold nanoparticles on thermal gradient generation and thermotaxis of E. coli cells in microfluidic device; Nithya Murugesan[4].

1.5. Thermal Camera

To measure temperature gradient we use a thermal camera, which is a device that uses infrared light to calculate temperature distribution and shows it in a form of colors or so. It helps (with the specialized FLIR software) to *see* temperature distribution in a surface and to know emissive temperature. We used ®FLIR E6 Infrared Camera.

1.6. Importance of thermal gradients over growth and distribution of E. coli bacteria

Microorganisms often have to navigate through their surroundings in search of nutrients and a better environment for survival. One of the important environmental cues microorganisms follow is temperature. Bacteria sense temperature differences and follow thermal gradients towards their favourable temperature which could help bacteria colonize certain regions.

The relation between temperature and bacteria growth has already been studied with different approaches as water content, energy available, and others as the effect of temperature on composition of fatty acids in *Escherichiacoli*. Variations in the temperature of growth and in the composition of the medium alter the proportions of individual fatty acids in the lipids of *Escherichiacoli*.[5]

2. Objectives

2.1. Main Objective

The main goal of this work was to study Heating-Ink based on Graphene to understand its properties, it is the intrinsic properties of the raw ink, the temperature generation dependence of morphology of the printed patterns when an electrical current is applied, and how to obtain specific temperatures due to resistivity of the printed patterns and voltage applied. Once Ink behavior is understood, it is possible to print ink patterns to fabricate growth cells in which we can control generated temperature, and then to fabricate an incubator, a device that carries growth cells and allows us to control the applied electrical power needed to generate our desired temperature gradients to study bacteria growth and distribution related to temperature.

2.2. Development of Methodology for Ink Deposition

Ink deposition was chosen because of its reliability and the less complexity, which leads to simplicity when studying the physical phenomenon that occurs in the device on its working time, and that allows us to craft a device with more specific properties too.

In order to achieve Ink deposition a lot of empiric tests are carried out to determine the best method to print lanes needed; among those test, we tried spraying a diluted version of the original ink with different solvents but any solvent we tried changed the properties of the ink so we discard spraying, the final method was direct applying with an spatula using white tape as template to mirror its thickness.

2.3. Design of Temperature Gradient Cells

By using squared glass cover-slips with a printed lane of heating ink we create heat on one side of the glass square and temperature varies along the material, giving as result a thermal gradient that can be controlled by regulating current induced to the heating lane.

2.4. Design of Incubator for Temperature Gradient Cells Evaluation of Bacteria Growth Distribution

Temperature gradient cells are taken just as surfaces to deposit, so in order to make them work they are clustered on a surface and connected to a power source of variable voltage and current. It is necessary to design a device that can carry on the squared cells and make them work properly.

3D CAD Software is used to design parts and the assembly that is needed to achieve a device capable of providing electrical energy, and a controlled environment for bacterial culture.

3. Experimental strategy

3.1. Characterization of Ink Properties

In order to achieve efficient and controllable thermal cells knowing the properties of the deposited ink is a must.

Some empiric tests were carried out in order to understand resistivity, conductivity, heating, and necessary dimensions for heating lines.

3.2. Temperature Gradient Generation on Glass Cover-Slips by Ink Deposition

Heat generation Inks are deposited on squared glass cover-slips on one of its edges so a lane is created, electrodes of silver based ink are deposited too and after that cover-slips are heated to cure.
Once ink is deposited conductivity, resistivity and heat generation are tested.

3.3. Design and construction of Temperature Gradient Generation Cells (TGGCs)

To achieve our goal of study bacteria distribution and division behaviour, it is needed to design and build specific tools as *growth cells (GCs)*, and *temperature gradient growth cells* (TGGCs) that fit on a special designed and built incubator.

GCs are made of Agar medium poured over a glass coverslip (22x22x1.17 mm) with special made molds to get 1 ml of volume over a 22x22 mm surface (squared films of 2 mm of thickness).

TGGCs are the same, but additionally a resistive ink lane (22x2.5 mm) is placed on the opposite face of the same glass coverslip on one end of that face, and then a silver nano-particles based conductive ink its placed on both sides of the resistive ink lane to act as electrical contacts, so we get a resistive heater on one end of the squared glass cover slip that acts as heat source to create the temperature gradient along the glass and Agar.

3.4. Design and construction of Incubator

More than one TGGC was analyzed each time we ran an experiment, and this was achievable thanks to the development of a special incubator. This incubator was designed as a 3D model made of multiple assemblable parts, those parts were cut from black acrylic sheets (of 3 mm thickness) by laser, and afterwards assembled using $CHCl_3$ to stick pieces together. Finally an electric circuit was placed into the incubator to regulate resistivity (so regulates input voltage too) for each squared hollow. We extruded a total of eight 22x22 mm squared hollows with a depth of 3mm, so we were able to insert the TGGCs there to bring them electric energy to act.

The electric circuit (Diagram 1) was based on two metal endings placed on different corners of the same edge of each hollow, and a potentiometer (variable resistance) of 5 kΩ for each hollow. This gave us a total of 16 endings (eight positive and eight negative) regulated by a potentiometer each two pair of terminals. This with the objective of being able to regulate temperature created on the TGGCs by controlling input voltage on them.

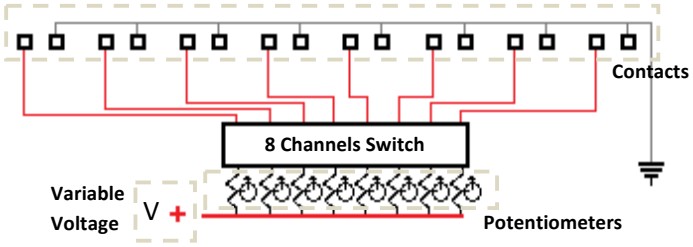


Diagram 1. Electric Circuit used to energize the slots of contacts for TGGCs.

3.5. Evaluation of growth and distribution of bacteria through temperature gradient

We were looking for the relation between temperature and growth behaviour on bacteria by adjusting parameters as temperature ranges, time periods and bacteria starting densities. By designing and building special equipment and then analyzing under a microscope quantity of bacteria for each LB-bacteria solution density, and according with their location on the TGGC compared with the thermal image taken for each calibrated TGGC.

4. Methodology

4.1. Characterization of Deposited Inks

Heating produced by deposited inks depend on length, width and thickness, so several combinations were deposited to understand proportions needed. By taking a specific length and varying thickness and width of deposited Ink., temperature and resistivity were measured for different deposited lanes that were under electrical current induction so printing becomes a standardized process to achieve heaters with the same rank of operation with electric current.

4.2. Design of Incubator

Incubator design consists of three different factors:

- Thermal Gradient Cells, which are the core of the incubator.
- Electric Circuit, needed to calibrate and to control temperature gradients along the glass-slips.
- Acrylic Structure, in which electrical circuit is encapsulated, cavities for thermal gradient cells to fit, and a covering that provides a controlled air volume with slits that allow bacteria gas residues to flow out the incubator cover box.

4.3. Medium for Culture/Growth

In order to analyze the growth of a bacteria population under any conditions a LB (*Luria Bertani*) solution is needed. LB is a nutritionally rich medium commonly used in microbiology. It is based on some peptides and casein peptones, some vitamins (including B vitamins), some trace elements and minerals (as nitrogen, sulphur, magnesium, etc).

With specific concentrations (2.5 g) of this medium in water (100 ml), a standard *growth medium solution* with a pH of 7.2 has to be obtained to later on, add a bacteria colony (as seed) and then incubate at 37 ºC in a shaking incubator at 250-300 rpm. As note: In order to have a sterilized medium, it is heated above 100 ºC and procedure of adding bacteria into the LB medium is made near the flame of a burner (less than 10 cm away from the flame) with previously sterilized material, as micropipette tips, tubes used as containers, etc.

To analyze bacteria growth, a solid medium is needed too. This is made based on a LB solution and adding some Algae (1.5 g for each 100 ml of LB solution) which produces a jelly-like substance called Agar, a medium that provides nutrients and water needed by bacteria to live and reproduce, and brings a solid surface very useful to analyze bacteria.

4.4. Preparation of Bacteria

To understand bacteria distribution at any kind of growth technique, and to ensure about the same quantity of bacteria on each experiment, it is needed to know the density of bacteria disperse in a LB medium (liquid). Achieving this is possible with an absorption spectrometer used at 600 nm of wave length, that is the wave-length needed to detect *E. coli* bacteria (turbidity), and starting from the aqueous LB-bacteria medium, some dilutions are measured; primal solution (1:1), 1:10, 1:100 and 1:1000 solutions. This standardizes our quantity of bacteria each time we repeat the experiments.

4.5. Characterization of Bacteria Colonies

Bacteria colonies are studied by growing them under specific conditions . First of all a well known method is implemented, it is a standard growth method which consists on the addition of LB-bacteria solutions over an Agar-LB based medium and settled on a traditional incubator (which is an isolated box with adjustable heater) at 37 ºC for 24 h. This allows us to observe the normal growth behaviour of bacteria for each dilution so we can compare that with temperature gradient growth and it helps to select which density of bacteria suits better for our experiments.

4.6. Optical Microscopy

Once we run an experiment and some bacteria are growth on our GCs, we observe under the microscope (10-40x of magnification) how much bacteria do we have. First of all we observe the growth-cells after letting bacteria divide for 24 h at 37 ºC in a conventional incubator and then we observe the temperature-gradient growth-cells to see its effect on bacteria division for different temperatures and different time periods.

5. Results

5.1. Characterization of Inks

Characterization of inks was carried out by following different experiments.
The first experiment was to print full surface of glass sample holders and measuring its heat generation at a given voltage and current. Width of deposited lanes were diminished on steps of ~1µm on every attempt to observe how its variation affects heating and resistivity of the samples.
After that, squared cover slips were taken as the standard, so lanes of 22 mm were the *specific length* to only vary width and thickness. Thickness is controlled only by the technique used, that in this case was based on direct spatula application limited by white tape thickness of 0.47 µm. Optimal width was determined by establishing the desired resistivity to a given voltage.

5.2. Characterization of Growth Cells

Growth Cells were built based on Agar Jelly that contains all nutriments needed for bacteria to grow, so lots of squared jellies were made and then exposed to different colonies with specific densities of bacteria.
So we observed that desired densities were between 0.0283 - 0.0324 (1:100) and 0.0008 - 0.0010 (1:1000) optical densities.

5.3. Fabrication of GCs and TGGCs

GCs are basically films of 2mm of thickness with a surface of 22x22 m made of Agar placed over a glass cover slip, but a mould was needed to achieve that shape and size.
To fabricate them a glass slip is placed at the bottom of each of four cavities of a mould and then 1 ml of liquid agar (it is liquid at around 70 ºC) is poured inside each of them too over the glass slip. Two moulds were used each time so we got eight 1 ml jellies with the desired shape.

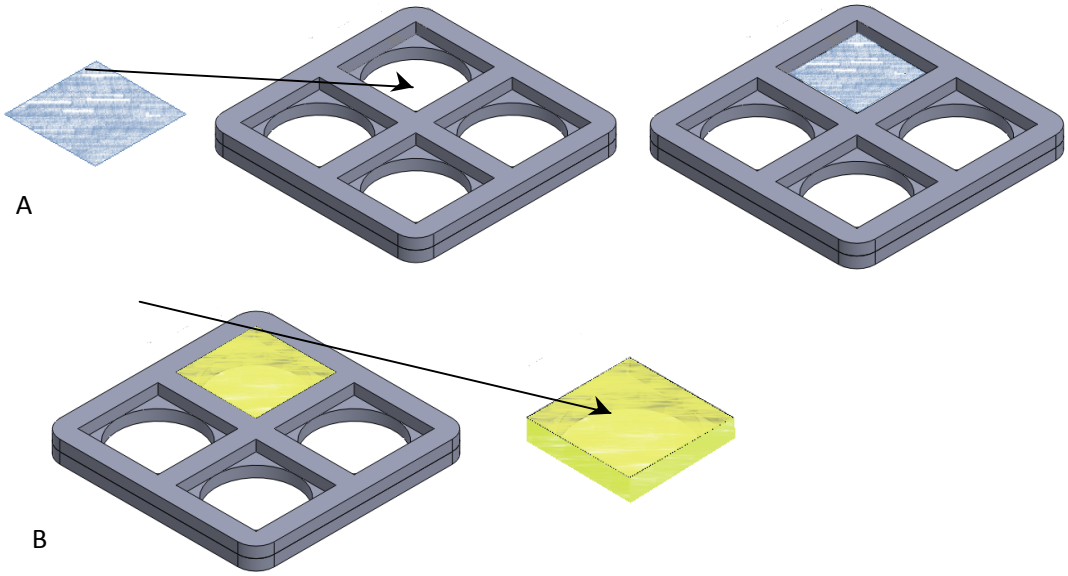


Fig. 3 - A: glass coverslip is placed on the mold; B: 1 ml of liquid Agar is poured over the glass slip, it is left over there for about 20 min to let it cold and solidify.

As it is shown in Fig.3, the process of making squared jellies was possible because of a special mold designed as a 3D model and then cut from acrylic of 3mm (it was made of to pieces and then glued together with $CHCl_3$ to make a one piece mold able to hold the glass slip and the liquid volume needed of Agar.

TGGCs are based on a glass cover slip with a printed lane of resistive ink and their respective printed contacts based on conductive ink that contains AuNP (Silver based Ink) as shown in Fig. 4.

Fig. 4 - A: glass coverslip; B: glass slip with the resistive ink printed on it; C: conductive contacts placed over the resistive ink.

5.4. Incubator

The incubator was made of a collection of pieces cut from black acrylic of 3mm of thickness, assembled with $CHCl_3$ as shown in the Fig. 5.

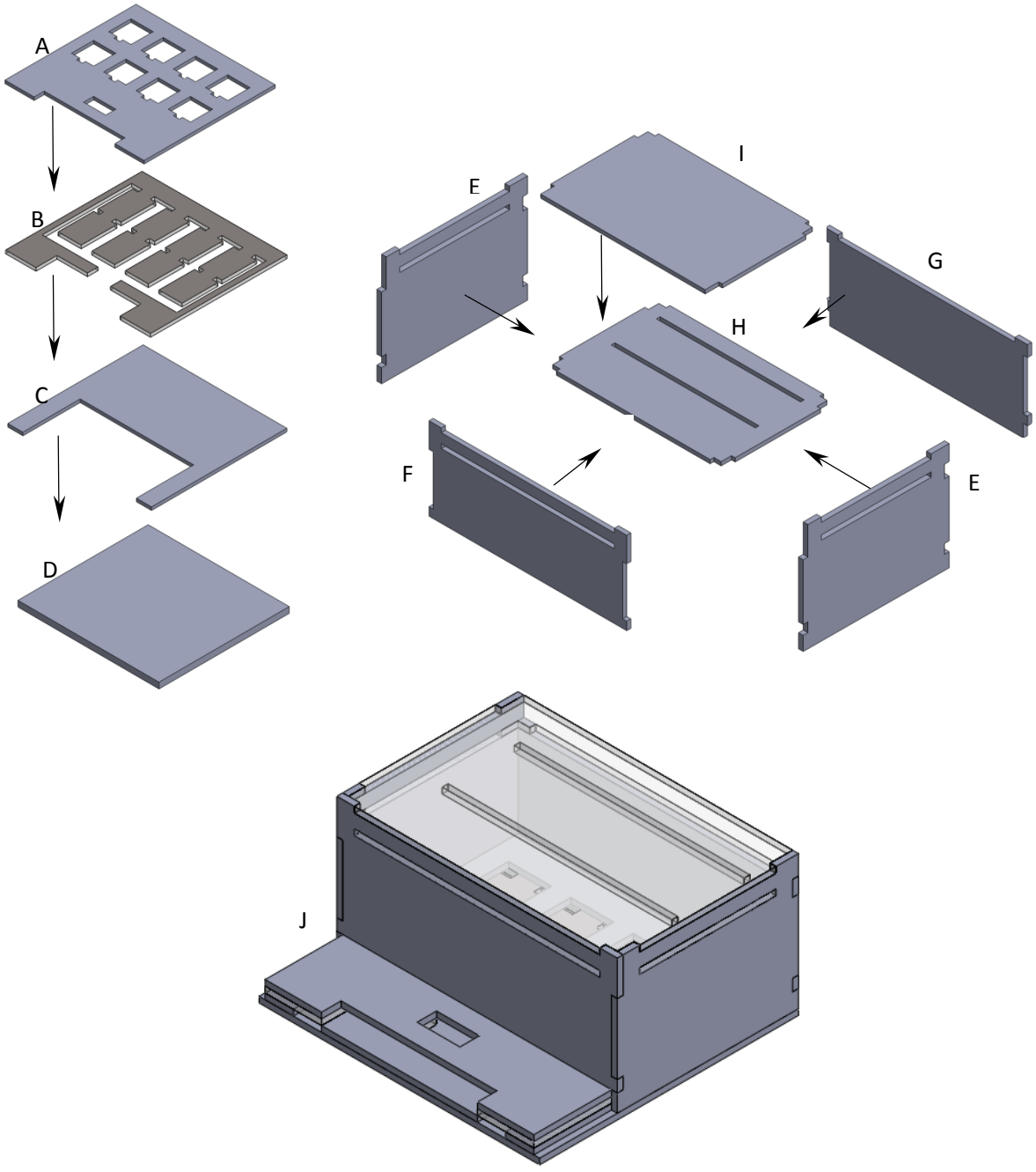
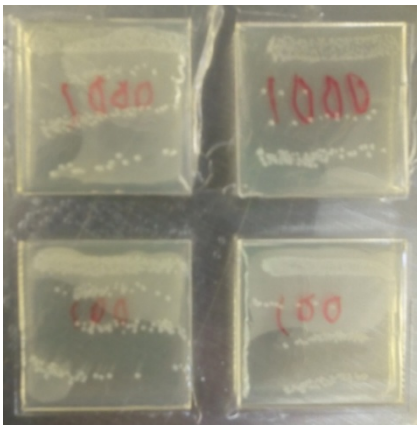


Fig. 5 - A: Surface of cavities; B: Electric circuit layer; C: Space layer; D: Base; E: Side walls of the covering; F: Frontal wall of the covering; G: Rear wall of the covering; H: First ceiling; I: Second ceiling; J: Assembled Incubator.

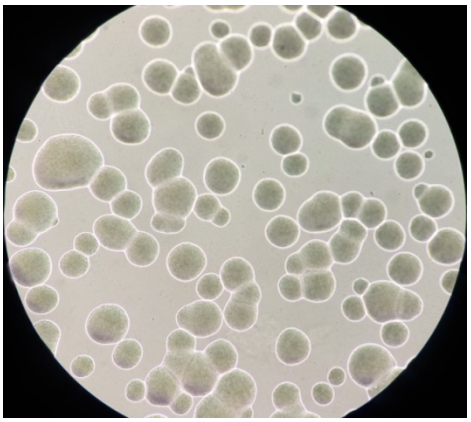
5.5. Characterization of GCs and TGGCs

To characterize GCs we poured 100 µl of LB-bacteria solution of different bacteria densities to observe bacteria growth under standard conditions, this is to place the squared Agar films into a Petri box and then placed inside a traditional incubator for 24 h.

Then we observed results shown in Pic. 1 and Img. 1.

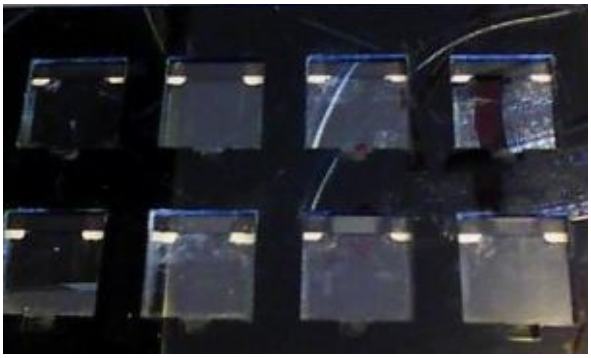


Pic. 1 - Picture of four of the Agar cells after traditional growth for densities of 1:100 and 1:1000

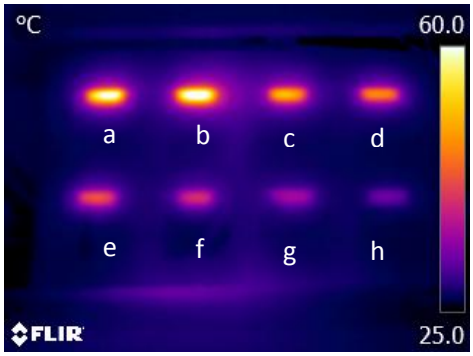


Img. 1 - Image of some colonies taken from an optical microscope camera at 10x magnification.

To characterize TGGCs different measurements (with a fixed bacteria density each time) were made taking a rank of temperatures going from 32 ºC to 60 ºC to know which density of bacteria was going to be used and to select a temperature for the TGGCs (shown in Pic. 2 and Img. 2)



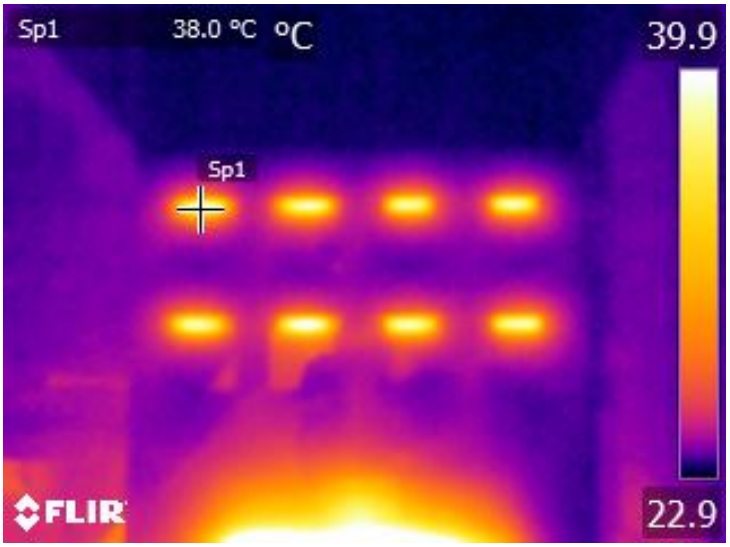
Pic. 2 - Picture of eight TGGCs placed over the Incubator.



Img. 2 - Image taken from FLIR Tools software, acquired with a E6 Infrared Camera.

Temperature of (a) was 60 ºC and it was decreased in steps of 4 ºC down to 32 ºC at (h).

After several experiments, first growth characterization was set at 38 ºC with optical densities of 1:100 and 1:1000. On Img. 3 is shown that all cells are at the same temperature (38 ºC).



Img. 3 - Image taken from FLIR Tools software, aquired with a E6 Infrared Camera. Temperature of all cells is 38 ºC.

5.6. Optical Densities of Bacteria Solutions

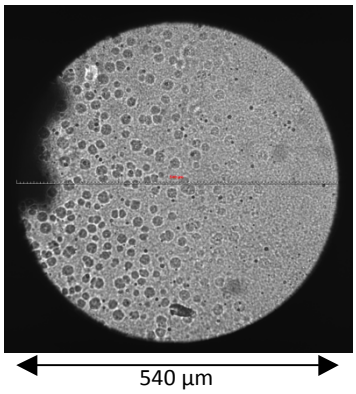
Optical densities of bacteria were measured by absorbance spectrometry (using a Cary 60 UV-Vis spectrometer from Aglient) and values obtained are shown in Tab. 1.

Dilutions	1:1	1:10	1:100	1:1000
Optical Densities	0.7714 - 0.9102	0.1524 - 0.2011	0.0283 - 0.0324	0.0008 - 0.0010

Tab. 3 - Optical densities are measured in absorbance units, and it is shown as a rank from the lowest to the highest measured densities.

5.7. Bacteria distribution over temperature gradients

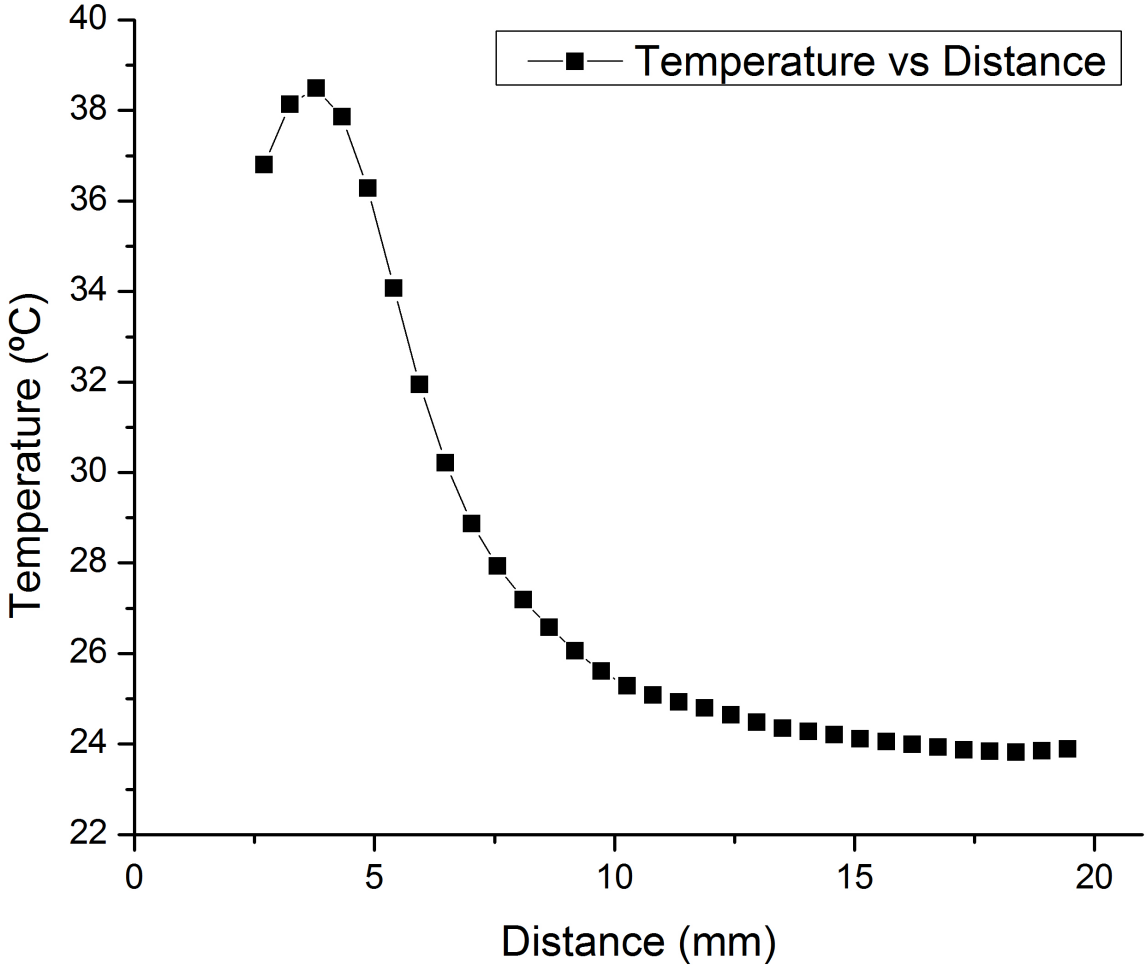
First of all, length of image given a 40x magnification on the optical microscope was made, to ensure a correct measurement of growth cells and temperature location. This is shown on Img. 4.



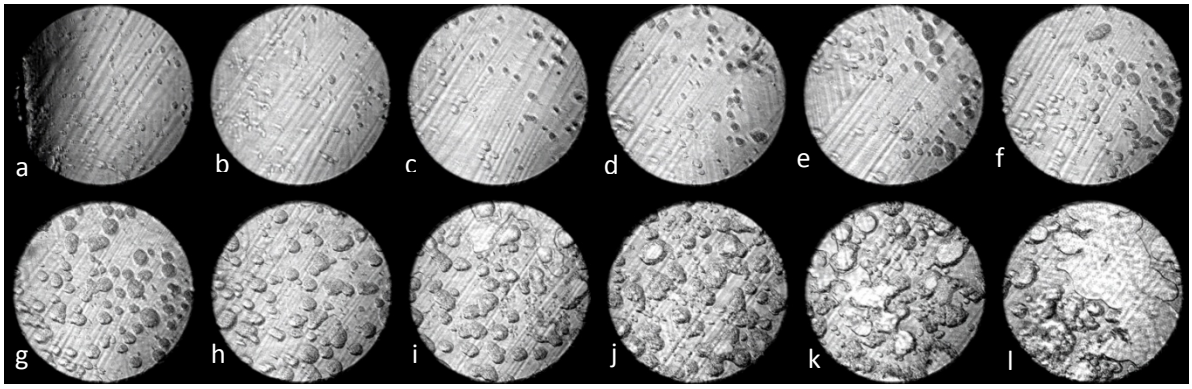
Img. 4.- Shows a sample measured by a calibrated ruler from side to side of the image taken from the optical microscope at 40x magnification..

Bacteria was analyzed in two stages, the first one was a collection of specific spots in which bacteria colonies were observed taking on count location (distance from the heating edge) and temperature given each spot. This results are shown in Graph.1 and Imgs.5-6.

Second stage was a collection of all images taken from the optical microscope from 2.5 to 19.5 mm. This is shown on Graph. 1 (as temperature distribution along the sample) and Imgs. 7-8 (where bacteria distribution is shown for 8 and 12 h of culture with a dilution rate of 1:100).

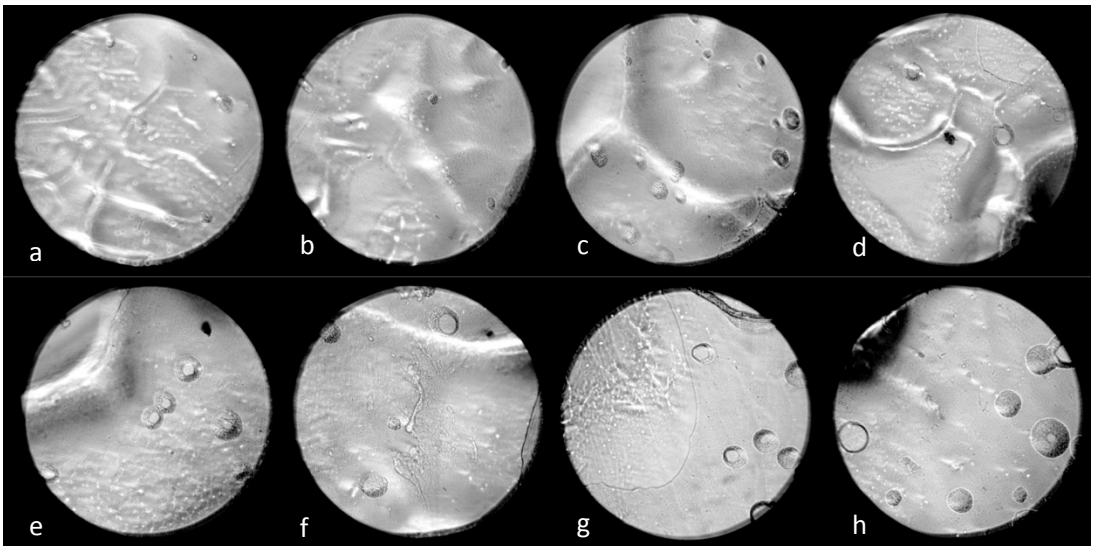


Graph. 1.- Shows the temperature distribution along the sample.



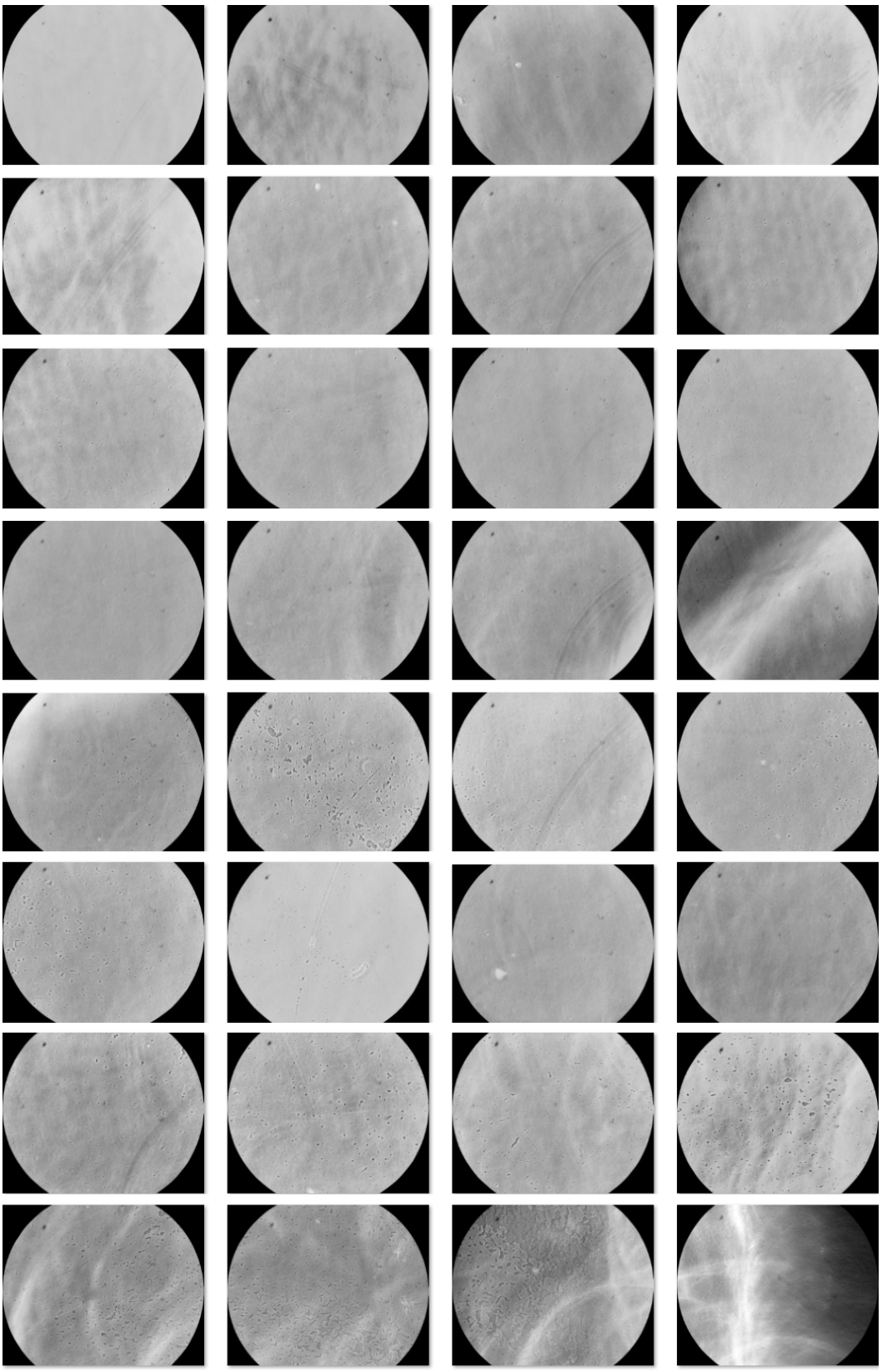
Img. 5.- Shows the bacteria distribution observed by optical microscopy, in some positions along the sample for a 1:100 dilution.
a: 21.60 - 27.00 mm@36.80 ºC; b: 37.80 - 43.20 mm@37.86 ºC; c: 54.00 - 59.40 mm@31.95 ºC; d: 70.20 -75.60 mm@27.93 ºC; e: 8640-9180 mm@26.05 ºC; f: 10.26-10.80 mm@25.08 ºC; g: 11.88 - 12.42 mm@24.64 ºC; h: 13.50 - 14.04 mm@24.28 ºC; i: 14.58 - 15.12 mm@24.11 ºC; j: 15.66 - 16.20 mm@23.99 ºC; k: 16.74 - 17.28 .mµm@23.87 ºC; l: 17.82 - 18.36 mm@23.82 ºC

In Img. 5 bacteria colonies are shown as round stains in **a - i** and as mostly amorphous stains in **j - l,** giving information about density of colonies over the sample and the variation of their size. This information is necessary to determine which concentration of bacteria is better to culture so we can observe as much colonies as possible.

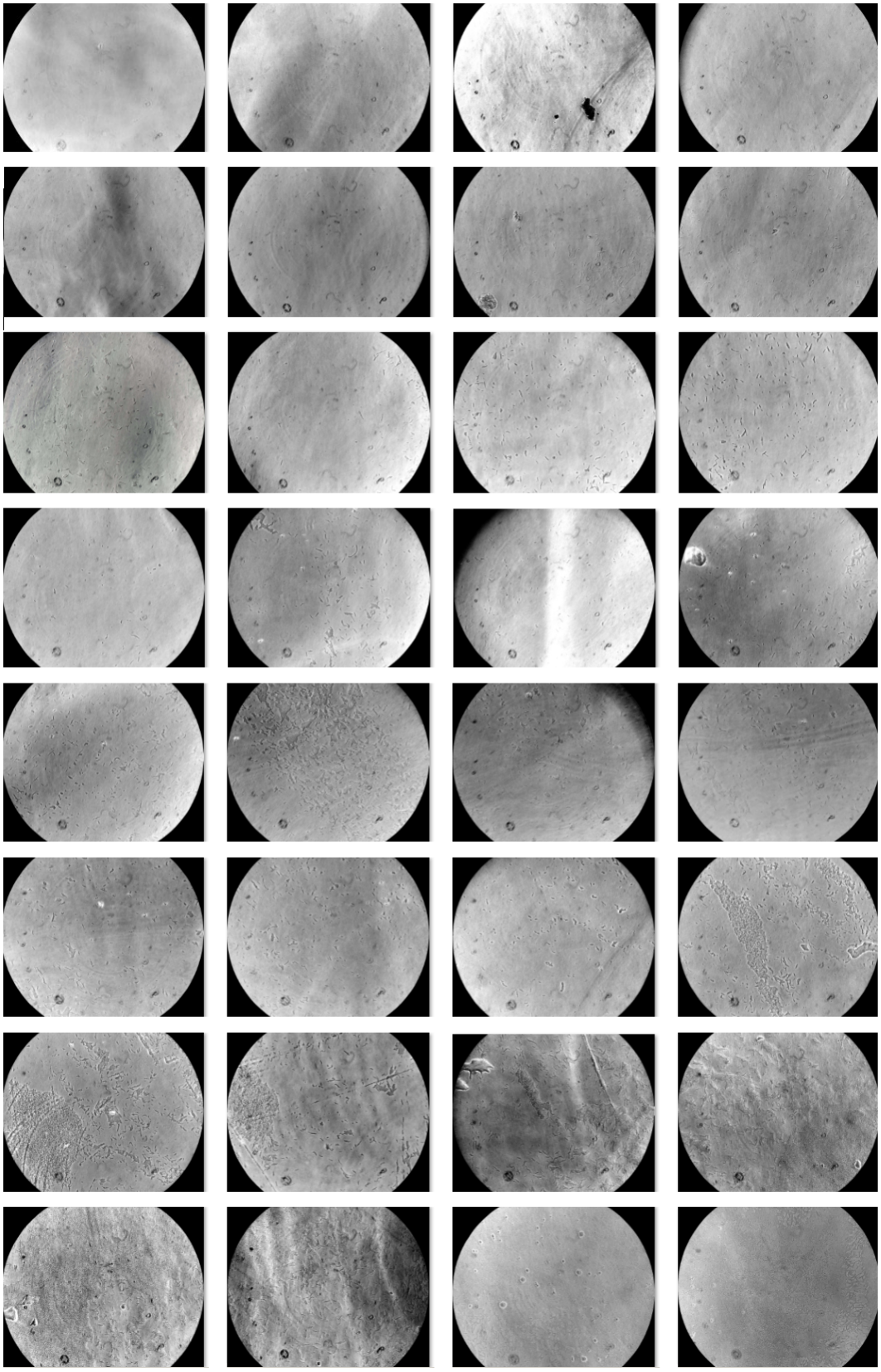


Img. 6.- Shows the bacteria distribution observed by optical microscopy, in some positions along the sample for a 1:1000 dilution.
a: 37.80 - 43.20 mm@37.86 ºC; b: 59.40 - 64.80 mm@30.21 ºC; c: 81.00 - 86.40 mm@26.58 ºC; d: 10.26 - 10.80 mm@25.08 ºC; e: 12.42 - 12.96 mm@24.48 ºC; f: 14.58 - 15.12 mm@24.11 ºC; g: 16.74 - 17.28 mm@23.87ºC; h: 18.90 - 19.44 mm@23.89 ºC.

In Img. 6 bacteria colonies are easy to observe but the quantity of them is not enough to notice the difference of growth as it is noticeable from figure **c** to **g** that represent a big section of the sample, but brings not much information about the effects of thermal gradients, therefore a dilution of 1:100 was set as optimal for our purposes.



Img. 7 - 32 pictures from 27 mm at 36.80 ºC to 19.44 mm at 23.89 ºC (see Graph. 1). For a dilution rate of 1:100 on a growth time of 8 h.



Img. 8 - 32 pictures from 27 mm at 36.80 ºC to 19.44 mm at 23.89 ºC (see Graph. 1). For a dilution rate of 1:100 on a growth time of 12 h.

Once we set our bacteria concentration some variations of culture period were used to understand how time affects bacteria distribution and to observe how the size of colonies depends just on the initial concentration, and to know if there is a phenomenon of organisms migration over the sample with time so we can differentiate effects of time from those that depend on temperature exclusively.

As shown in Img.7, 8 h are not enough to develop big colonies due to slower reproduction rate noticeable by the quantity of bacteria groups, which are mostly isolated bacteria over the lower temperature gradient (close to 23.89 ºC). In Img. 8 we can observe the same effect as on Img. 7 for the section of lower temperature gradient, but a bigger increase of colony sizes for higher temperatures (close to 36.80 ºC). As both experiments (8 and 12 h) show similar results, we know that temperature is playing an important role on how bacteria colonies develop.

6. Discussion

It was observable that a dilution of 1:100 (Img. 5) was better than 1:1000 (Img. 6) to notice the effect of thermal gradients over bacteria populations due to a bigger density of the colonies on the samples. Therefore, a dilution of 1:100 was set as constant to vary then the time of culture, which was of 8 h (Img. 7) and 12 h (Img. 8). Because of the taken images it is evident that 12 h is a better period of incubation, the density of colonies is bigger and the effects of thermal gradients over bacteria population are easier to analyze.

Ratkowsky et. al[6],[7] showed that there is a behaviour dependent of temperature for speed of growing and the life time of bacteria, it is not only the temperature the important topic but the time in which division occurs. Even if bacteria has its most efficient development at 37 ºC, when temperature is higher division occurs faster but life time of individuals decay, and in the other hand, when temperature is lower than optimal division occurs slower but lifetime is extended.
This is what we observed on this work, because even if bacteria had optimal conditions most of individuals never divided due to a short life time and far from optimal conditions populations of bacteria trended to be more extensive.

7. Conclusions

Our experiments show that bacteria population size has a direct relation between temperature and time of growth, so we concluded that bacteria can reproduce better at 37 ºC but they die faster than colonies that are cultured below that temperature so those does not have the same opportunity to develop a huge population.

References

[1] Ho, Kuan-I; Boutchich, Mohamed; Su, Ching-Yuan; Moreddu, Rosalia; Marianathan, Eugene Sebastian Raj; Montes, Laurent; Lai, Chao-Sung (2015). "A Self-Aligned High-Mobility Graphene Transistor: Decoupling the Channel with Fluorographene to Reduce Scattering"
[2] Kipphan, Helmut (2001), Handbook of print media: technologies and production methods (Illustrated ed.), Springer, pp. 130–144, ISBN 3-540-67326-1.
[3] Inkjet Printing of High Conductivity, Flexible Graphene Patterns; Ethan B. Secor,Pradyumna L. Prabhumirashi, Kanan Puntambekar,Michael L. Geier,and Mark C. Hersam
[4] Effect of gold nanoparticles on thermal gradient generation and thermotaxis of E. coli cells in microfluidic device; Nithya Murugesan, Tapobrata Panda, Sarit K. Das, Springer Science&Business Media New York 2016 - Biomed Microdevices (2016).
[5] Effect of temperature on the composition of fatty acids in escherichia coli; Allen G. Marr, Jhon L. Ingraham, Department of Bacteriology, University of California, Davis, California.
[6] Relationship Between Temperature and Growth Rate of Bacterial Cultures; D. A. Ratkowsky, June Olley, T. A. Mcmeekin, A. Ball, Division of Mathematics and Statistics and Division of Food Research, Commonwealth Scientific and Industrial Research Organization; and Department ofAgricultural Science, University of Tasmania; Hobart, Tasmania, Australia 7000.
[7] Model for Bacterial Culture Growth Rate Throughout the Entire Biokinetic Temperature Range; D. A. Ratkowsky, R. K. Lowry, T. A. Mcmeekin, A. N. Stokes, R. E. Chandler, Department of Agricultural Science, University of Tasmania, and Division of Mathematics and Statistics, Commonwealth Scientific and Industrial Research Organization, Hobart, Tasmania, Australia 7000, and Division of Mathematics and Statistics, Commonwealth Scientific and Industrial Research Organization, Melbourne, Victoria, Australia 3168.