Escherichia coli's response to shock by SDS and ethanol mixed solution

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Abstract

Hand sanitizers are often used to clean our hands by supposedly removing or killing the bacteria on them. Hand sanitizers are usually composed of ethanol or triclosan as active agents. Ethanol needs to be at a concentration above 60% and be exposed during at least 15 seconds (1) to kill a majority of bacteria. Whereas for SDS, with a concentration higher than 1%, less than 2% of *E.coli*, a commonly found bacteria, survives (2). In this study, we compare the antibacterial properties of ethanol and sodium dodecyl sulfate (SDS) separately and in different proportions when mixed with a culture of *Escherichia coli* bacteria. Spectrophotometry is used to obtain growth curves for the bacteria and punctual absorbance values of the culture. Our results indicate the important slowing of *E. coli* growth and maybe even bacterial death when in presence of as little as 40% of ethanol. They also show the relative inefficiency of SDS at a concentration of 0.01% for 10 minutes. Our results can lead to important reevaluation of currently used antibacterial agents. We can also account for the fact that ethanol is commonly used to disinfect surfaces or objects and that each product generally uses only one active agent.

Introduction

For many people bacteria means dirty and disease, so to fight against microbes more and more people use hand sanitizers. This kind of solution is said to be completely antibacterial and we wanted to see if they are really efficient. Based on a study about *Bactericidal effects of triclosan in soap both in vitro and in vivo* (3) published in 2015, we decided to study the composition of hand sanitizers and other types of detergent. We noted that triclosan is not the only detergent used in this kind of hygiene product : alcohol is also really present. Indeed, ethanol is an alcohol which is often used as an antibacterial to sterilize the lab. Ethanol denatures bacterial protein by effectively killing them. In other cases, particularly in detergents, sodium dodecyl sulfate (SDS) is used to kill bacteria (5)(6). We compared the efficacy of both chemicals on *Escherichia coli,* a bacterium often used as a model organism. *E.coli* is commonly found in the environment around us and has the particularity to replicate every 20 minutes at 37°C. We asked ourselves the following question : How does E.coli react to a ten minute shock by an SDS and ethanol mix?

Experimental design for SDS and ethanol mix

To respond to the question, we first conducted a preliminary experiment *(which can be found in the supplementary materials*) to determine our optimal concentration of ethanol and SDS. We did not want to kill the bacteria but wanted to see a response. Indeed, after our preliminary experiment we decided to test different ratios of ethanol and SDS in a mixed solution. However, our initially determined concentrations might have been too important or too small to observe a response.

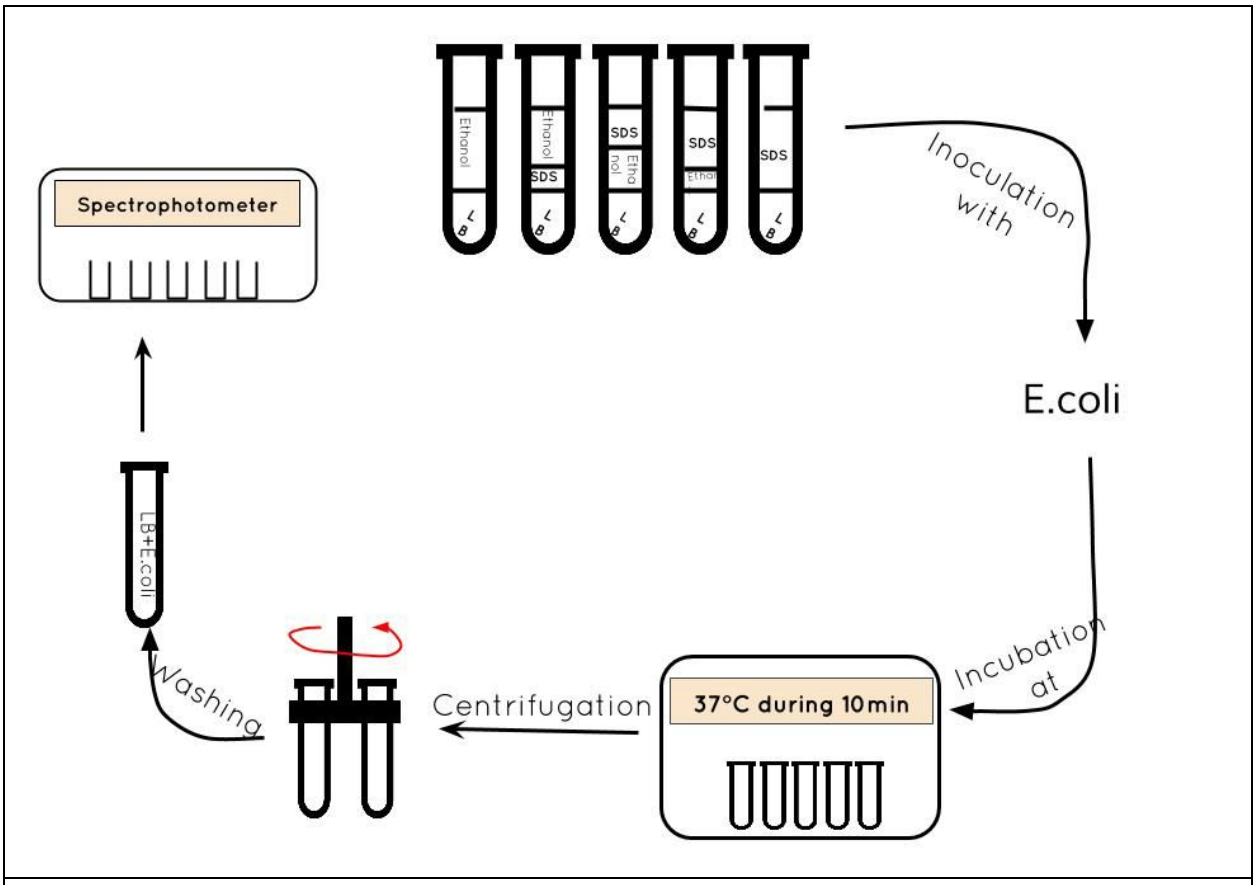


Figure 1 : Experimental protocol
Ratios: 1 : 100% ethanol / 0% SDS; 2 : 75% ethanol / 25% SDS; 3 : 50% ethanol / 50% SDS; 4 : 25% ethanol / 75% SDS; 5 : 0% ethanol / 100% SDS; 6 : Only LB

From this analysis, we chose a 40% concentration of ethanol and a 0,01% concentration of SDS. We prepared solutions of different ratios of ethanol to SDS: 100/0, 75/25, 50/50, 25/75 and 0/100. Liquid LB was used for the dilutions in 2,5 mL eppendorfs. We inoculated these different altered liquid media with our night culture of *E.coli* bacteria and incubated in a static incubator at 37°C for ten minutes. These ten minutes attempted to replicate our hand's or a surface's bacteria's exposition to a detergent. During this time, bacteria began their growth if they manage to resist, or were killed by the detergent. These concentrations and conditions were chosen because they resemble the ones that hand sanitizers are used in.

We then removed the bacteria from the altered media by centrifuging them for 1 minute at 9.5 rcf to separate the two and resuspended the cells in liquid LB. A spectrophotometer was used to measure the absorbance of 1 mL of this solution at three different time points: t=0h, t=60min, and t=120min.

Absorbance values according to ratio

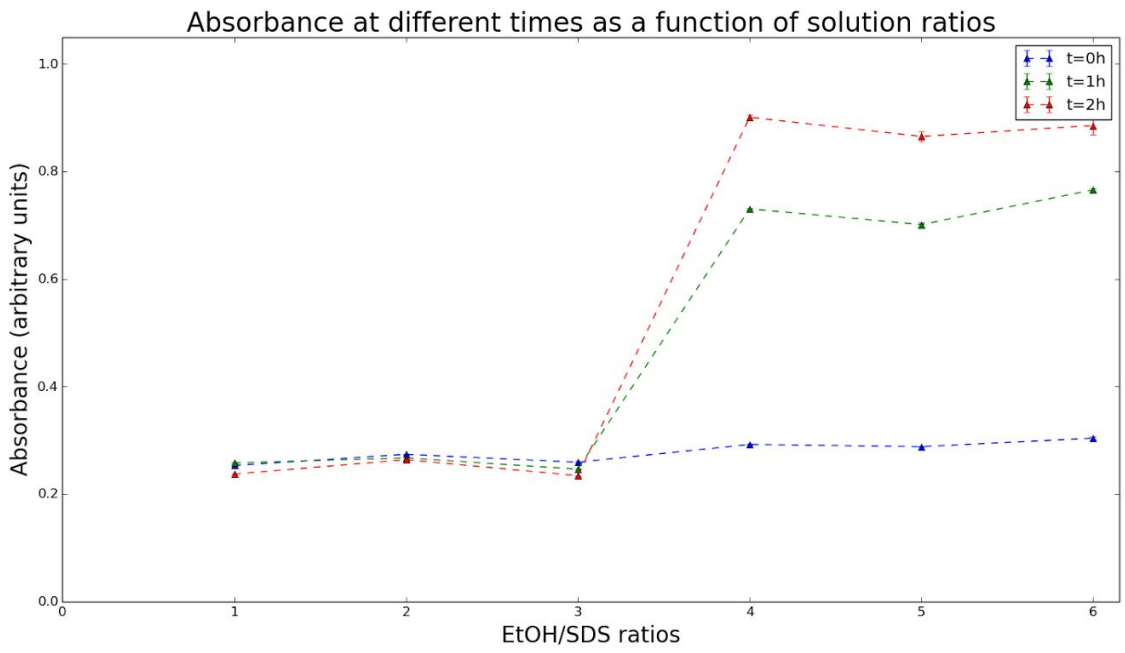


Figure 2 : Absorbance at different time as function of solution ratios
Ratios: 1 : 100% ethanol / 0% SDS; 2 : 75% ethanol / 25% SDS; 3 : 50% ethanol / 50% SDS; 4 : 25% ethanol / 75% SDS; 5 : 0% ethanol / 100% SDS; 6 : Only LB

This figure shows our absorbance in function of different ratios of SDS and ethanol after we resuspended the bacteria in LB. We removed the ethanol and SDS of our E.coli solution and put them in LB media to favorize their growth again. At t = 0 h, the absorbance is between 0.25 and 0.3 arbitrary units (a.u.); absorbance is almost the same for each ratio. At t = 1 h, the absorbance is around 0.25 a.u for the first three ratios and for the last three ratios, the absorbance is between 0.7 and 0.75 a.u. Finally, at t = 2h, the absorbance of the first three ratios is again the same but for the last three ratios, the absorbance is between 0.85 and 0.9 a.u.

We can also notice that for the first and third ratios, the absorbance decreases over time. Absorbance values should have been increasing if the bacteria were still alive or should have remained constant if the bacteria were dead or no longer growing. We can account for this because of the corrosive properties of SDS or because of errors in manipulation such as not resuspending or consistently pipetting for the different absorbance values. More explanations for this can be found in the discussion.

Furthermore, we observe that bacteria do not grow any more after the shock when the proportion of ethanol is equal or higher than the SDS proportion whereas when the ethanol proportion is higher than the SDS proportion, bacteria continue to grow. This observation could

be explained by the supposition that the SDS concentration is not sufficient to kill all bacteria in 10 minutes or that this timelapse is too short for the SDS to become effective.

Ethanol, a more efficient detergent than SDS

From the results above, we could say that the ratio 50/50 is the most efficient as it is the one that has the lowest absorbance values and its values decrease over time. However, the values are very close to the ratio 100/0 and knowing that SDS can be quite corrosive the first ratio would be a better a hand sanitizer.

Our initial hypothesis was that two commonly used antibacterial agents used together would lead to less bacterial growth. However, the 50/50 and the 100/0 ratios are very similar. Our concentration of ethanol might have been too high to notice a differentiated response. Therefore, ethanol is a more efficient detergent than SDS in our experimental conditions. Meaning with 10 minute exposure, at a 40% concentration of ethanol and 0.01% concentration of SDS, incubation at 37°C and time points every hour for three hours, ethanol is more effective.

Our results are interesting in the light of other publications in the field of microbiology and toxicology. In the study *Growth inhibitory and lethal effects of ethanol on Escherichia coli* (7), it is noted that stationary cell populations were more resistant than exponential ones to ethanol. Our preliminary results indicate that the population was not in exponential phase *(Supplementary Materials*) therefore possibly altering our results. This study also shows the independence of ethanol's response from temperature, meaning that our conclusions might be also relevant for other temperatures. We can see in the can also see in other articles (8), that there is sometimes an interfering effect of the solvent on bacterial growth, allowing us to question if liquid LB, a very rich medium, was optimal for experimentation.

Sources of error and perspectives

Though our results are scientifically relevant, the patterns we observed and interpreted might have been misled by the noise in our data. In fact, the decrease and increase that we observed for different detergent ratios could be caused by biological noise, the natural variations between organisms within a specie. Errors in manipulation could have occurred and general variability due to the people manipulating cause us to push for more experiments on the topic. We could redo our experiment with more technical replicates, preparing the different altered ratio media independently. More frequent measurements of absorbance could be taken, giving us a more precise response curve. Biological replicates would also be necessary to minimize the biological noise. We could innoculate separately two identical solutions, use another night culture or use another strain of bacteria to see the true influence of the ethanol/SDS ratio. We are also highly aware of the bias of our preliminary experimental results and analysis on our second experiment. We could redo this experiment several times to make sure that the right concentrations were chosen.

We could continue this experiment by testing other concentrations and other detergents. One of our initial concerns was the different behavior between *E.coli* and other bacteria or fungi on our hands. We could therefore cultivate bacteria found on our hands by making a hand print

on a solid LB media petri dish and redo our experiment with this bacteria. We could also create experimental conditions that are closer to reality. Anti-bacterial agents are usually used on surfaces, like our hands or countertops, and we tested in liquid media conditions. We could create modified growth media with less nutrients and the active agents, either ethanol, SDS, triclosan or a mix, and grow our bacteria on a solid form. We could also try to recreate the experimental protocol used by the MicroChem Lab in Texas, USA to test hand sanitizers according to the Food and Drug Administration (9).

Acknowledgements

We would like to thank Tamara, Aïmen, Ivan, and our mentor Nicholas Garcia. We would also like to thank our sponsors, the Center for Research and Interdisciplinarity and the OpenLab.

Ressources

(1) **Scienceline.ucsb.edu,. "UCSB Science Line".** N.p., 2016. Web. 21 Feb. 2016

(2) **Researchgate.net,. "Fig. 1. SDS Sensitivity Of E. Coli Tat Mutant Strains**. Strains MC4100 (...". N.p., 2016. Web. 21 Feb. 2016.

(3) **Bactericidal effects of triclosan in soap both in vitro and in vivo,** S. A. Kim, H. Moon, K. Lee and M. S. Rhee. 2015. Antimicrobial Chemotherapy

(4) **Curing Action of Sodium Dodecyl Sulfate on a Proteus mirabilis R+Strain**, MUNEMITSU TOMOEDA and All. JOURNAL OF BACTERIOLOGY, Dec.1974,

(5) **Effects of Treatment with Sodium Dodecyl Sulfate on the Ultrastructure of Escherichia coli**, C. L. Woldringh. J Bacteriol. 1972 Sep.

(6) I**nteraction between sodium dodecyl sulfate and membrane reconstituted aquaporins: A comparative study of spinach SoPIP2;1 and E. coli** AqpZ. Jesper S. Hansen Biochimica et Biophysica Acta (BBA) - Biomembranes Volume 1808, Issue 10, October 2011

(7) **Growth inhibitory and lethal effects of ethanol on Escherichia coli** , Y. Nakamura, T. Samada. Journal of Biotechnology and Bioengineering. 1987 Apr. Web. 15 Feb. 2016.

(8) **Effect of various solvents on bacterial growth in the context of determining MIC of various antimicrobials**, T. Wadhwami et al., Internet Journal of Microbiology, 2013. Web. 11 Feb. 2016.

(9) **Hand sanitizer testing services**, Jan. 2011, MicroChem Laboratory Procedures. Web. 20 Feb 2016.

Supplementary materials

→ Supplementary methods

- ***Cultures***

We cultured E.coli strain MG1655-2014.
Ethanol was 96% but we approximated it at 100%, the SDS solution was concentrated at 1%

- ***Preliminary Experiment : determining the ideal concentration of detergents***

The goal of the preliminary experiment is to determine which concentration of ethanol and SDS provoked a response of the bacteria without killing them all in order to have a better final experiment.

To do this we chose to look at 5 different concentrations of SDS and ethanol plus the positive and negative controls. The concentration of ethanol were 70%, 60%, 50%, 40% and 30% while for the SDS it was 0.1%, 0.01%, 0.001%, 0.0001%, 0.00001%. The positive control were eppendorf with only ethanol and only SDS while the negative control were only LB. We inoculated E. coli at 37° for 10 min in the different concentration of ethanol and SDS. Then, we wash the cells and add LB and put them in the 96 well-plate and put them in the TECAN spectrophotometer for 2h and a measure every 10 min in order to see a growth curve. However the cell did not grow as expected as we might not have put enough cells in the tube at first (10uL for an eppendorf of 1mL), or they might not have been in exponential phase.

- **Methylene blue staining attempt**

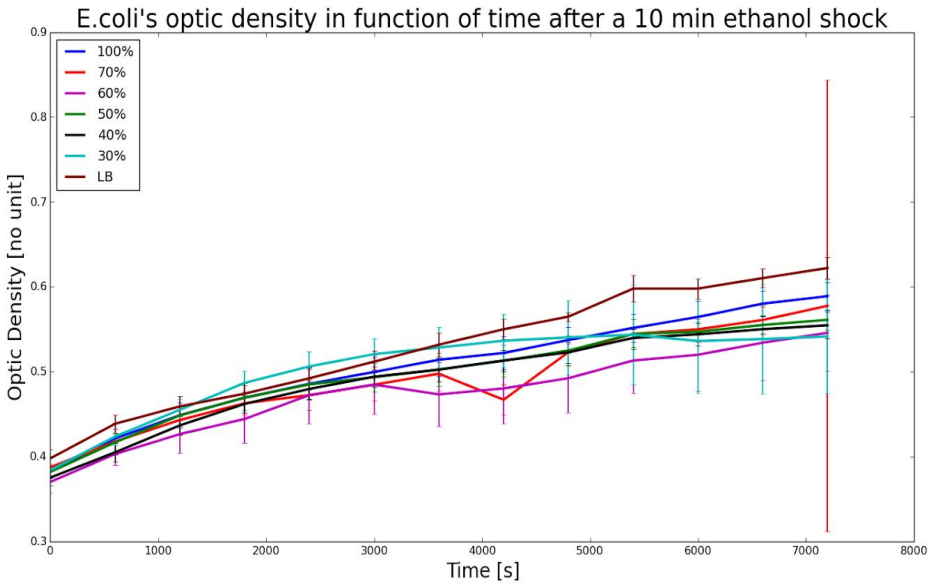
We try to make a ratio between bacteria dead or not. To do that we used the blue coloration, with methylene blue to color our bacteria.
So, under the hood, thanks to a loop we put a bacteria solution on a microscope slide. We wait for the drying of the solution to fix bacteria on the slide. Then, outside the hood, we cover our bacteria solution with methylene blue. We wait for 5 minutes and removed the blue thanks to distilled water and look at the slide under microscope.

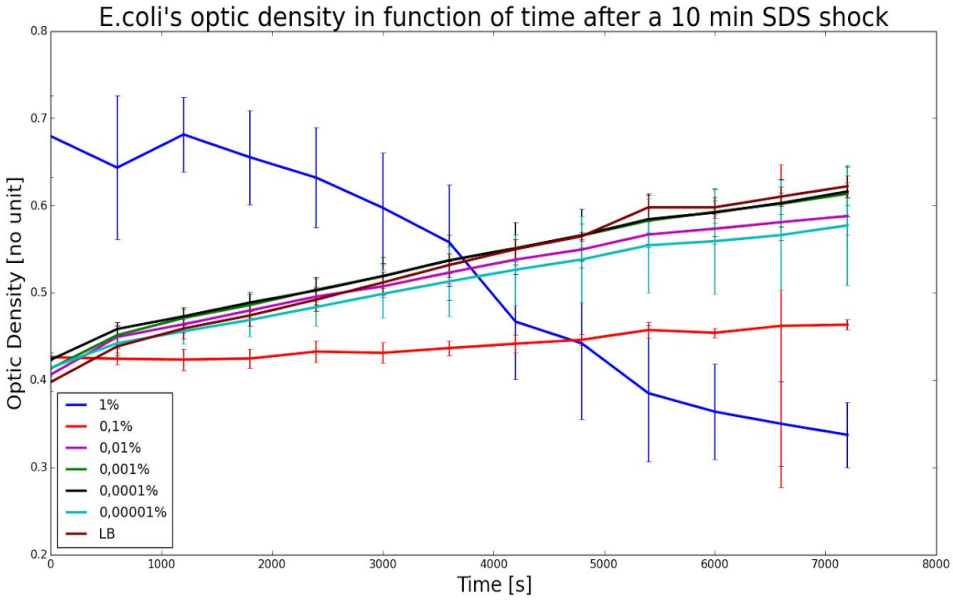
→ Supplementary results

- **Preliminary Experiment results**

We did a first preliminary experiment, using a 96-well plate and measured the optical density continuously for 10 hours. We observed that the absorbance values did not change and therefore thought that there were either no bacteria in the wells or that there was no growth. After reviewing our lab notebook, we realized that we started an *E. coli* night culture from the end of a frozen culture, possibly containing only glycerol and dead cells. We therefore redid the experiment the next day with a new culture and the following results come from that experiment.

The different curves show the different concentration values that were tested. There were 4 technical replicates per concentration.





The role of this experiment being to determine the optimal concentrations for our following experiment, we carefully analyzed our data. We wanted to choose concentrations of ethanol and SDS that did not entirely kill or stop the growth of *E. coli* but we also wanted to observe a response to the altered environment. According to the FDA and several publications, 60% is the minimal concentration of ethanol to kill most bacteria on a surface. We therefore chose a 40% concentration because the response is different from bacteria in normal LB and does not kill the bacteria. Following the same reasoning, we chose an intermediate concentration of 0.01%. The trends can more easily be distinguished without the error bars but we have kept them here to show the variability of the results.

We formulate concerns pertaining to the overall trends of the OD curves that do not have the exponential shape usually observed in growth curves for a similar time frame. This could have biased our second and primary experiment.

- **Raw data files from our primary experiment**

For ethanol :

```
#!/usr/bin/python
# coding=utf8

#import the library
import numpy as np
import matplotlib.pyplot as plt

time = np.loadtxt('Ethanol_average.txt', usecols= [0]) #open the file and load the columns [0] which is the first

#import of the mean of each concentration of alcohol
E0 = np.loadtxt('Ethanol_average.txt', usecols= [1])
E1 = np.loadtxt('Ethanol_average.txt', usecols= [2])
E2 = np.loadtxt('Ethanol_average.txt', usecols= [3])
E3 = np.loadtxt('Ethanol_average.txt', usecols= [4])
E4 = np.loadtxt('Ethanol_average.txt', usecols= [5])
E5 = np.loadtxt('Ethanol_average.txt', usecols= [6])
N = np.loadtxt('LB_average.txt', usecols= [1])

#standard deviation import
sd0 = np.loadtxt('Ethanol_STDEV.txt', usecols= [1])
sd1 = np.loadtxt('Ethanol_STDEV.txt', usecols= [2])
sd2 = np.loadtxt('Ethanol_STDEV.txt', usecols= [3])
sd3 = np.loadtxt('Ethanol_STDEV.txt', usecols= [4])
sd4 = np.loadtxt('Ethanol_STDEV.txt', usecols= [5])
sd5 = np.loadtxt('Ethanol_STDEV.txt', usecols= [6])
sdn = np.loadtxt('LB_STDV.txt', usecols= [1])


plt.xlabel('Time [s]', fontsize = 20) #name of x axis
plt.ylabel('Optic Density [no unit]', fontsize = 20) #name of y axis
plt.title("E.coli's optic density in function of time after a 10 min ethanol shock", fontsize = 26) #title of the graph

plt.errorbar(time,E0, yerr=sd0, color='b', linewidth=1.5) #draw of the error#
plt.errorbar(time,E1, yerr=sd1, color='r', linewidth=1.5)
plt.errorbar(time,E2, yerr=sd2, color='m', linewidth=1.5)
plt.errorbar(time,E3, yerr=sd3, color='g', linewidth=1.5)
plt.errorbar(time,E4, yerr=sd4, color='k', linewidth=1.5)
plt.errorbar(time,E5, yerr=sd5, color='c', linewidth=1.5)
plt.errorbar(time,N, yerr=sdn, color='maroon', linewidth=1.5)
```

```
plt.plot(time,E0, label='100%', color='b', linewidth=2.5) #draw the curve
plt.plot(time,E1, label='70%', color='r', linewidth=2.5)
plt.plot(time,E2, label='60%', color='m', linewidth=2.5)
plt.plot(time,E3, label='50%', color='g', linewidth=2.5)
plt.plot(time,E4, label='40%', color='k', linewidth=2.5)
plt.plot(time,E5, label='30%', color='c', linewidth=2.5)
plt.plot(time, N, label='LB', color='maroon', linewidth=2.5)
plt.legend(loc="upper left") #location of the legend
plt.show()
```

For SDS

```
#!/usr/bin/python
# coding=utf8

import numpy as np #import the library
import matplotlib.pyplot as plt

time = np.loadtxt('Ethanol_average.txt', usecols= [0]) #open the file and load the columns [0] which is the first

S0 = np.loadtxt('SDS_average.txt', usecols= [1])
S1 = np.loadtxt('SDS_average.txt', usecols= [2])
S2 = np.loadtxt('SDS_average.txt', usecols= [3])
S3 = np.loadtxt('SDS_average.txt', usecols= [4])
S4 = np.loadtxt('SDS_average.txt', usecols= [5])
S5 = np.loadtxt('SDS_average.txt', usecols= [6])
N = np.loadtxt('LB_average.txt', usecols= [1])


sd0 = np.loadtxt('SDS_STDEV.txt', usecols= [1])
sd1 = np.loadtxt('SDS_STDEV.txt', usecols= [2])
sd2 = np.loadtxt('SDS_STDEV.txt', usecols= [3])
sd3 = np.loadtxt('SDS_STDEV.txt', usecols= [4])
sd4 = np.loadtxt('SDS_STDEV.txt', usecols= [5])
sd5 = np.loadtxt('SDS_STDEV.txt', usecols= [6])
sdn = np.loadtxt('LB_STDV.txt', usecols= [1])

plt.xlabel('Time [s]', fontsize = 20) #name of x axis
plt.ylabel('Optic Density [no unit]', fontsize = 20) #name of y axis
plt.title("E.coli's optic density in function of time after a 10 min SDS shock", fontsize = 26) #title of the graph

#plt.errorbar(time,S0, yerr=sd0, color='b', linewidth=1.5) #draw of the error#
#plt.errorbar(time,S1, yerr=sd1, color='r', linewidth=1.5)
#plt.errorbar(time,S2, yerr=sd2, color='m', linewidth=1.5)
#plt.errorbar(time,S3, yerr=sd3, color='g', linewidth=1.5)
#plt.errorbar(time,S4, yerr=sd4, color='k', linewidth=1.5)
#plt.errorbar(time,S5, yerr=sd5, color='c', linewidth=1.5)
```

```
#plt.errorbar(time,N, yerr=sdn, color='maroon', linewidth=1.5)

plt.plot(time,S0, label='1%', color='b', linewidth=2.5) #draw the curve
plt.plot(time,S1, label='0,1%', color='r', linewidth=2.5)
plt.plot(time,S2, label='0,01%', color='m', linewidth=2.5)
plt.plot(time,S3, label='0,001%', color='g', linewidth=2.5)
plt.plot(time,S4, label='0,0001%', color='k', linewidth=2.5)
plt.plot(time,S5, label='0,00001%', color='c', linewidth=2.5)
plt.plot(time,N, label='LB', color='maroon', linewidth=2.5)
plt.legend(loc="lower left") #location of the legend
plt.show()
```

- **Python programs used to analyse raw data and create graphs**

```
#!/usr/bin/python

import matplotlib.pyplot as plt
import numpy as np
import pylab as pl

#Importing data from our txt file
D=np.loadtxt("spectro_thurs.txt")

#We calculate the mean and standard deviation per row
M = np.mean(D, axis=1)
S = np.std(D, axis=1)

#we create an arbitrary list for the x axis
X=[1,2,3,4,5,6]

#we plot the different biological replicates with error bars
plt.errorbar(X,M[0:6],yerr=S[0:6], label='t=0h', fmt='^--')
plt.errorbar(X,M[6:12],yerr=S[6:12], label='t=1h', fmt='^--')
plt.errorbar(X,M[12:19],yerr=S[12:19], label='t=2h', fmt='^--')

plt.xlabel("EtOH/SDS ratios", fontsize = 20)
plt.ylabel("Absorbance (arbitrary units)", fontsize = 20)
plt.title("Absorbance at different times as a function of solution ratios", fontsize = 24)
plt.savefig("fig1.png")
plt.axis([0, 6.15, 0, 1.05])
plt.legend()
plt.show()
```

- **Code for the arduino program**

```
// Example testing sketch for various DHT humidity/temperature sensors
// Written by ladyada, public domain

#include "DHT.h"
```

```
#define DHTPIN A0    // what pin we're connected to

// Uncomment whatever type you're using!
//#define DHTTYPE DHT11   // DHT 11
#define DHTTYPE DHT22   // DHT 22  (AM2302)
//#define DHTTYPE DHT21   // DHT 21 (AM2301)

// Connect pin 1 (on the left) of the sensor to +5V
// Connect pin 2 of the sensor to whatever your DHTPIN is
// Connect pin 4 (on the right) of the sensor to GROUND
// Connect a 10K resistor from pin 2 (data) to pin 1 (power) of the sensor

DHT dht(DHTPIN, DHTTYPE);

void setup()
{
        Serial.begin(9600);
        Serial.println("DHTxx test!");

        dht.begin();
}

void loop()
{
        // Reading temperature or humidity takes about 250 milliseconds!
        // Sensor readings may also be up to 2 seconds 'old' (its a very slow sensor)
        float h = dht.readHumidity();
        float t = dht.readTemperature();

        // check if returns are valid, if they are NaN (not a number) then something went wrong!
        if (isnan(t) || isnan(h))
        {
        Serial.println("Failed to read from DHT");
        }
        else
        {
        Serial.print("Humidity: ");
        Serial.print(h);
        Serial.print(" %\t");
        Serial.print("Temperature: ");
        Serial.print(t);
        Serial.println(" *C");
        }
}
```