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MEGBI - Middle East
Genetics and Biotechnology
Institute

مركز أبحاث للجينات والتقنية البيولوجية

MEGBI-APP (Antibiotics Pilot Plant)

Report 2023

Penicillin, Ampicillin, and Aspirin production and quantification

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1 Preface:

The pharmaceutical industry is a significant pillar of any healthcare system, and its scope covers drug discovery, development, manufacturing, and marketing. In light of the COVID-19 outbreak, medicine accessibility was challenged worldwide, putting many people's lives at risk. High levels of drug consumption and insufficient local pharmaceutical manufacturing remain a big challenge for the pharmaceutical supply chain and healthcare system. Localizing the manufacturing of drugs, and their ingredients in residence country, is vital to protect any country's healthcare system and enhance its readiness for emerging outbreaks beyond COVID-19 like several others sudden outbreaks which could spread at any time.

Local production of pharmaceuticals plays a vital role in maintaining resilience of national healthcare systems, especially when it comes to facilitating access to needed medicines and decreasing exposure to imports and international supply chains. Pharmaceutical companies typically operate in both national and international markets, through which they are subjected to specific regulations and healthcare policies that govern drug manufacturing, approval, marketing, and sales. These legislations are different from one country to another, depending on the healthcare challenges they face, and could directly influence the discovery, development, manufacturing, and sales of new drugs.

In any world countries generally, and in Lebanon specially ,drugs are the main product needed by the consumer to treat even to prevent several diseases. And the main type of drug to be used is antibiotics (penicillin, ampicillin...), even Aspirin which is the most widely sold over-the-counter drug.

For Lebanon, there are not enough studies even local industries to produce even the basic drugs or medicines like these mentioned above, for this reason the import still the ideal solution to secure the locally need. According that, we as AECENAR, and specially as MEGBI institute, we try to find and even to produce these medicines locally by developing a bioreactor which can produce this types of drugs .

References:

2 Part I: Aspirin

Aspirin Qualification

Here we can find the price list of chemicals needed for testing:

Aspirin quality control standard tests according to the Ph.Eur.							
Test	Reagent	Quantity per experiment	Quantity ordered	Price	Reference	Missing Apparatus	
IR absorption	Potassium bromide	300-400 mg				IR Spectrophotometer, hydraulic press	
Color test	Calcium hydroxide	0.5 g	500 g	\$63.53	Biosynth		
Color test	2-Nitrobenzaldehyde solution	0.05 mL	0.25 kg	\$50.82	Biosynth		
Appearance of solution	Reference solution B9	For comparison	100 mL	\$107.70	Reagecon		
Related substances	Phosphoric acid					HPLC	
Related substances	Acetonitrile					HPLC	

Heavy metals	Thioacetamide solution	1.20 mL	25 g (19.7 mL)	\$69.00	Sigma		
Heavy metals	Buffer solution	2 mL	1 L	\$63.49	Reagecon		
Heavy metals	Lead standard solution	10 mL of 1 ppm	100 mL of 100 ppm	\$127.42	Reagecon		Note: needs to be in water
Loss on drying	Diphosphorus pentoxide	Amount depends on size of desiccator	500 g	\$154.00	Sigma	vacuum desiccator, weighing bottle	
Sulphate ash	Silica gel					crucible, muffle furnace, desiccator	
Assay	Phenolphthalein	As an indicator	50 g	\$44.84	VWR		
Total				\$680.80			

Finalized Price List

In order to work according to priorities, the first involves ordering large amounts of salicylic acid and acetic anhydride. As for the aspirin qualification, due to the specific apparatus that is required, we have decided to move forward with conducting a melting point test and therefore

References:

purchasing that apparatus. As for the remaining tests, those will be done at specialized laboratories, such as the university labs listed by the MOPH in order to register and certify a drug. In addition, this table includes components that are missing from the lab but are vital to the projects we are conducting, both now and in the future. Finally, we have also included the reagents required for phenylacetic acid production.

Title	Reagent	Quantity ordered	Price	Reference	Total
Aspirin production	Acetic anhydride	5 L	\$150.00	VTC (VWR)/in stock	\$150.00
Lab Equipment	Separatory funnel (1000 mL)		\$32.00	VTC	\$713.00
	Complete Buchner filtration apparatus (1000 mL)		\$150.00	VTC	
	Stand		\$16.00	VTC	
	Capillary tubes	100 units	\$5.00	VTC	
	Separatory funnel (500 mL)		\$20.00	City Med Lab	
	Safety goggles	3 units	\$18.00	City Med Lab	
	Fire Extinguisher	2 kg	\$12.00		
	Distillation (Vacuum)		\$90.00	Amazon	
	Shipping:		\$40.00		

	Melting apparatus	point		\$200.00	Alibaba	
	Shipping:			\$130.00		
PAA production	Methyphenyl acetate		1 kg	\$210.00	VTC	\$357.00
	Sodium hydroxide (2M)		1 kg	\$5.00		
	Ether		2.5 L	\$125.00	VTC	
	Na ₂ SO ₄		1 kg	\$17.00	VTC	
Total				\$1,220.00		\$1,220.00

Aspirin Optimization

Introduction

With the arrival of the melting point apparatus, the sample that had originally been produced on 25/11/2022 during the aspirin production protocol was tested for its melting point. Its range was approximately 114-116oC. In light of the impurities that have lowered the melting point, trials were conducted varying different parameters to both test the effect these parameters have on yield as well as on purity.

References:

Results

Trial	Volume	Molarity of Acid	Recrystallization solvent	Reaction time	Mass of Product	% Yield	FeCl ₃ test	Melting Point
1	5 mL acetic anhydride	1M (sulfuric acid)	warm water	10 min	1.96g	75.1%	+	120.6-121.4 °C
				10min	-		+	121.5-124.0 °C
				10min	-		+	120.8-124 °C
				15min	1.56g	60%	+	125-127 °C
				15min	1.21g	46.3%	+	126.7-130.5 °C
2	5 mL acetic anhydride	6M	water	10 min	1.60g	61.3%	+	117.7-119.6 °C
					2.42g	92.7%	+	121-128 °C
					1.07g	41.15%	+	123.7-125.8 °C
3	5 mL acetic anhydride	6M	ethanol and water	10 min	0.85g	32.6%	-	118.6-119.7 °C
			ethanol and cold water	15 min	0.68g	26.6%	+	119.5-122.1 °C
			ethanol and warm water	10min	0.88g	33.7%	+	125.3-128.2 °C
			ethanol and warm water	15 min	0.37g	14.1%	+	126.5-128 °C
			ethanol and warm water	15min	0.94g	36%	+	125.7-128.8 °C
			ethanol (10ml) and warm water	15min	0.65g	24.9%	+	121-122 °C
			ethanol 5%, distilled water at room temperature	15min	1.28g (unkown residue milky-oily mass)	49.1%	+	119.4-123.1 °C
				15min		79.6%	+	

			ethanol (10ml) and warm water		2.08g (98% concentrated acid)			
4a	1.56 mL acetic anhydride 1.29 mL acetic acid	1M	water	10 min	1.85g	70.9%	+	119.2-120.9 °C
4b	1.56 mL acetic anhydride 1.29 mL acetic acid	6M	water	10 min	2.37 g	90.8%	+	108.0-108.5 °C
5	5 mL acetic anhydride	6M	water	2 hr	-	-	-	Some melted at around 90 °C. Finished melting at 118 °C.
6	5 mL acetic anhydride	6M	water	20 min	2.39 g	91.5%	-	120.0-120.7 °C
7	1.56 mL acetic anhydride 3.44 mL acetic acid	6M	water	10 min	1.57g	60.2%	+	114-116 °C

References:

8*	5 mL acetic anhydride	6M	water	10 min	1.80g	69.0%	-	114-116 °C
9	5 mL acetic anhydride	6M	Room temp distilled water	10 min	2.97g	114%	+	114-123.3 °C
	4 ml acetic anhydride	6M	- ()	15 min	1g	40%	-	132-134 °C

*Using Biosynth salicylic acid. Based on visual appearance alone, the crystals are much larger than those of Xilong Scientific, the other supplier of salicylic acid. Seeing as the large scale production would utilize the salicylic acid purchased from Biosynth, we decided to conduct a trail using it.

Discussion

The theoretical yield of aspirin is 2.61g for excess acetic anhydride and 2g of salicylic acid. This was determined based on the following calculation: mass of aspirin = 180 g/mol (molar mass of aspirin) x 2 g salicylic acid / 138 g/mol (molar mass of salicylic acid).

Trial 2 was repeated a second time due to the positive ferric chloride test for the first sample. The second sample had a much higher yield and tested negative in the ferric chloride test.

The result of trial 5 was a yellowish putty, as though the product it contained had burnt. This might be due to the extended exposure to heat, or it might be due to the small quantity used within a large round bottomed flask. It was not possible to determine its exact mass due to its dampness, nor did it fit any of the characteristics of aspirin. For continuity purposes, it was included within the table along with its ferric chloride test and had its melting point crudely measured.

As hot water induces the hydrolysis of aspirin into acetic and salicylic acids, trial 9 was designed to observe whether using room temperature water would lead to an increase in the purity. However, as evident from its sizable mass and ferric chloride test, it prevented the salicylic acid from being washed away completely, meaning this was not a suitable step to take.

Trial 4 was designed based on the following patent: <https://patents.google.com/patent/US3235583A/en>. This patent describes how an 18% molar excess of acetic anhydride is sufficient to arrive at a good yield of aspirin. Based on this quantity, the following calculations were made:

$$n_{\text{acetic anhydride}} = 1.18 n_{\text{salicylic acid}} = 1.18 \times 2 \text{ g} / 138 \text{ g/mol} = 0.0165 \text{ mol}$$

$$V_{\text{acetic anhydride}} = n \times MM / d = 0.0165 \text{ mol} \times 102 \text{ g/mol} / 1.08 \text{ g/mL} = 1.56 \text{ mL}$$

In order to dilute the reaction mixture, it was calculated using the proportion that for 140 g of salicylic acid, 95 g of acetic acid were used. This calculation netted that for 2 g of salicylic acid, 1.36 g of acetic acid should be used, which equates to a volume of 1.29 mL.

The ferric chloride test indicated that both trials 4a and 4b contained salicylic acid. One possible reason behind this is the combined volume of the solvents is approximately 3 mL, and the increased concentration relative to the other trials may have prevented the salicylic acid from being washed away completely. Trial 7 was therefore designed to account for this possibility, yet its result was also positive for salicylic acid. This may imply that a repetition is required. Regardless, this allows for the opportunity to conserve the usage of the reactants.

The melting point is relatively low for all the trials regardless of whether they tested positive or negative for the ferric chloride test. It was also measured after leaving the samples to dry overnight in the refrigerator. To attempt to purify 4b, two trials for a third recrystallization were conducted. 0.5g of crude aspirin were used. The first trial involved adding 2 mL of ethanol and 15 mL of water and heating until all aspirin had dissolved. The second trial involved adding 17 mL of warm water only. Both trials resulted in samples that still tested positive in the ferric chloride test, with a melting point that was not significantly different from the parent material.

References:

Trial	Solvent	Agitation	Mass obtained	Melting Point
4b	2mL ethanol and 15mL water	No	0.39 g	108.0-108.5
4b	17mL water	No	0.40 g	107.1-107.8

Conclusive Remarks:

Overall, using an increased concentration of sulfuric acid has a positive effect on results by significantly increasing the yield. This is expected due to its catalytic activity expediting the reaction and driving it closer to completion. However, most samples had a relatively low melting point compared to the reported melting point of pure aspirin (135°C), as well as the melting point of medicinal aspirin (~130°C). Medicinal aspirin contains impurities such as maize starch, cellulose powder, methacrylic acid, and other coating reagents or those that aid in the absorption of aspirin. As such, it is expected that its melting point is depressed.

Following steps include using toluene as a recrystallization agent instead of water. This would preclude the hydrolysis of aspirin. Extra recrystallization steps may also be conducted on the samples obtained, particularly those that tested negative for the ferric chloride test.

Noting the inconsistencies across the two tests in trial 2, in order to ensure validity and reproducibility of these tests, it is worth repeating each trial three times

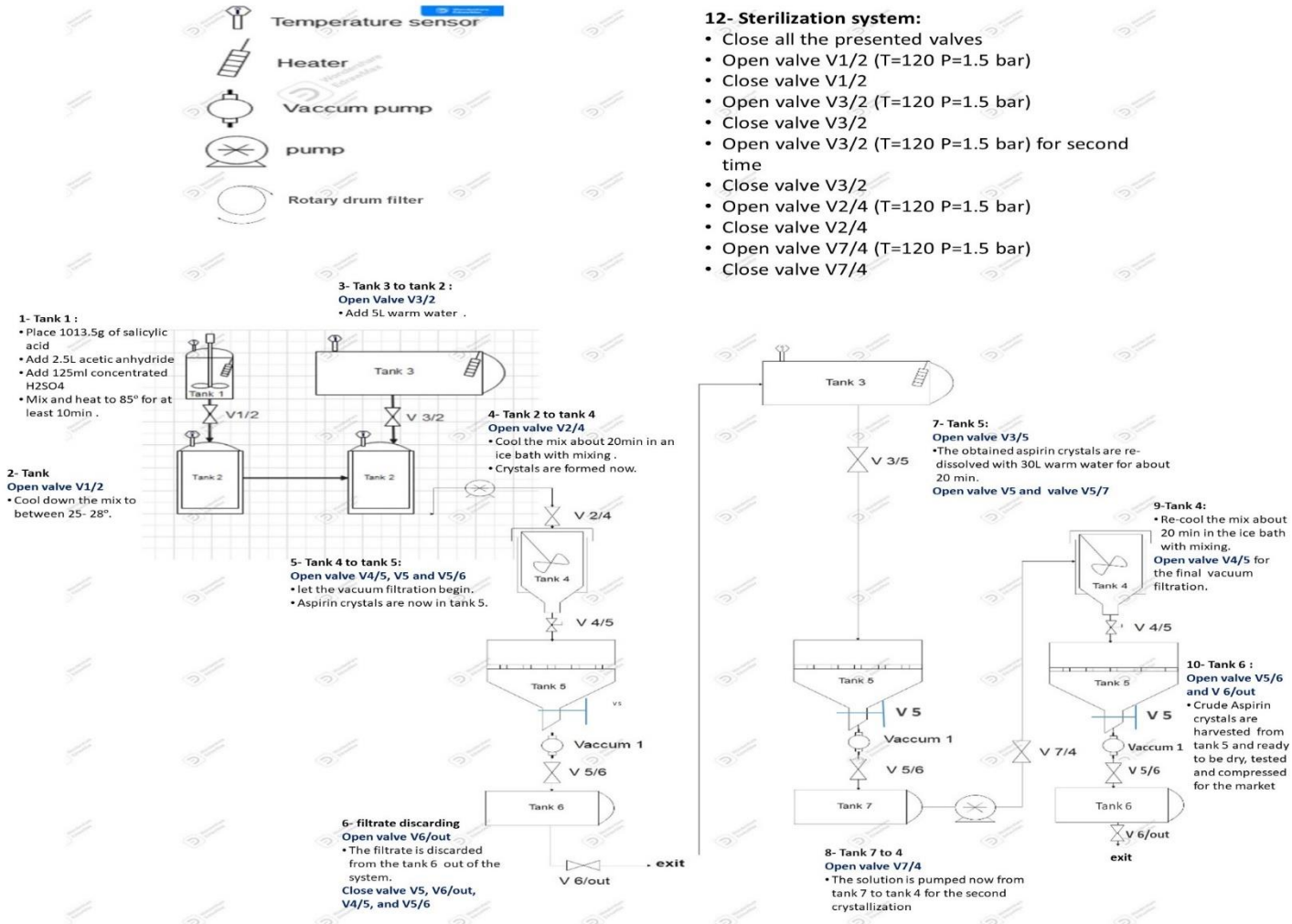


Figure 1 : Dynamic view of pilot plant aspirin production system

3 Part II: Penicillin

3.1 Penicillin Production and Quantification May2023

Trial 2:

according to the sensitivity test :

_F1(Aqueous phase after the organic solvent extraction)

References:

_F2(Organic phase After PB extraction)

_F3(the final solution obtained after organic solvent extraction)

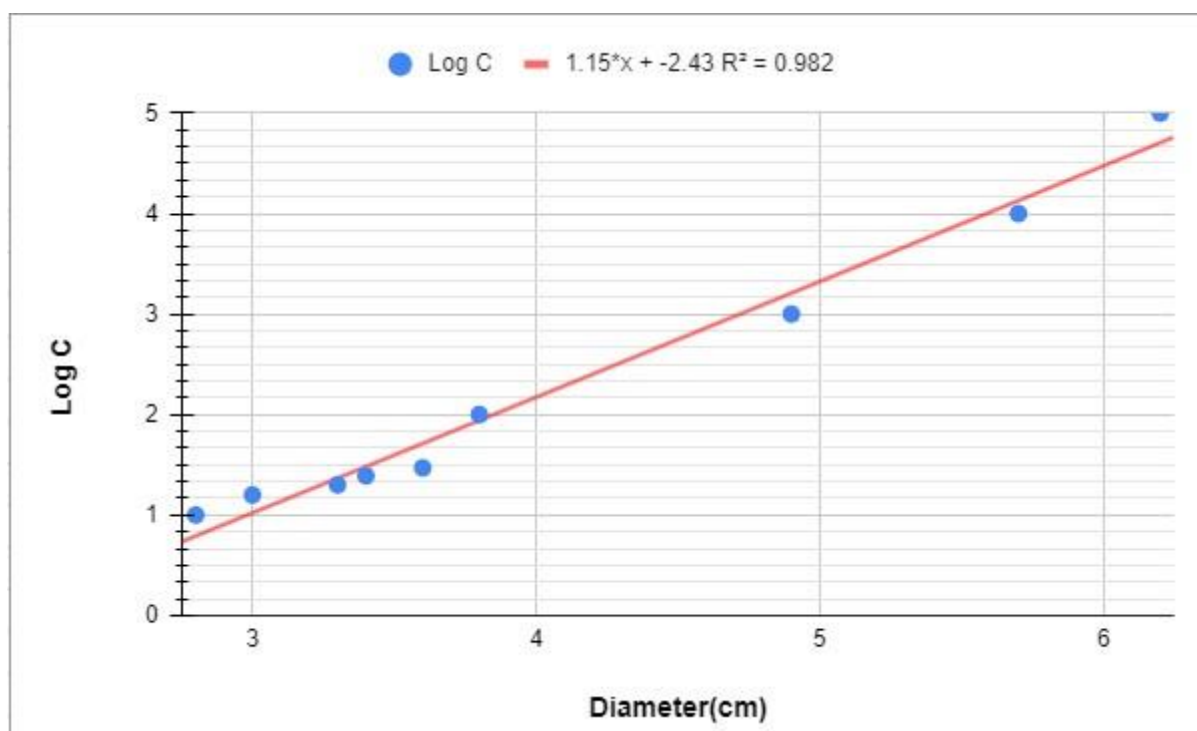
Penicillin Quantification (May 2023)

The procedure for quantification is the same as that mentioned before using disc diffusion method leading to a calibration curve helping to calculate the concentration of our crude PenG obtained from F1 and F3 solution:

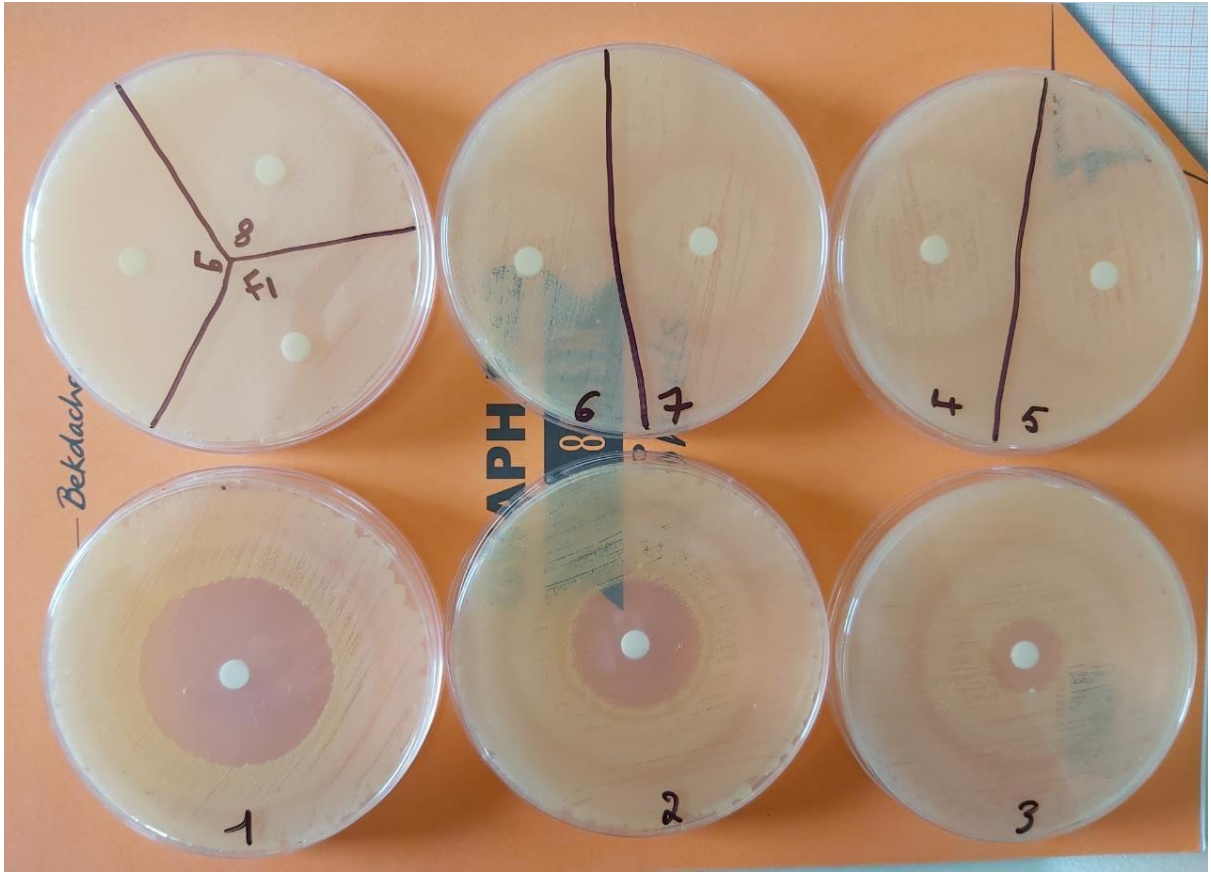
Quantification of Crude PenG obtained from F1 crystallization:

Table1 : Measurements of diameters of bacterial growth inhibition zones for different concentrations of standard dilute commercial penicillinG and F1.

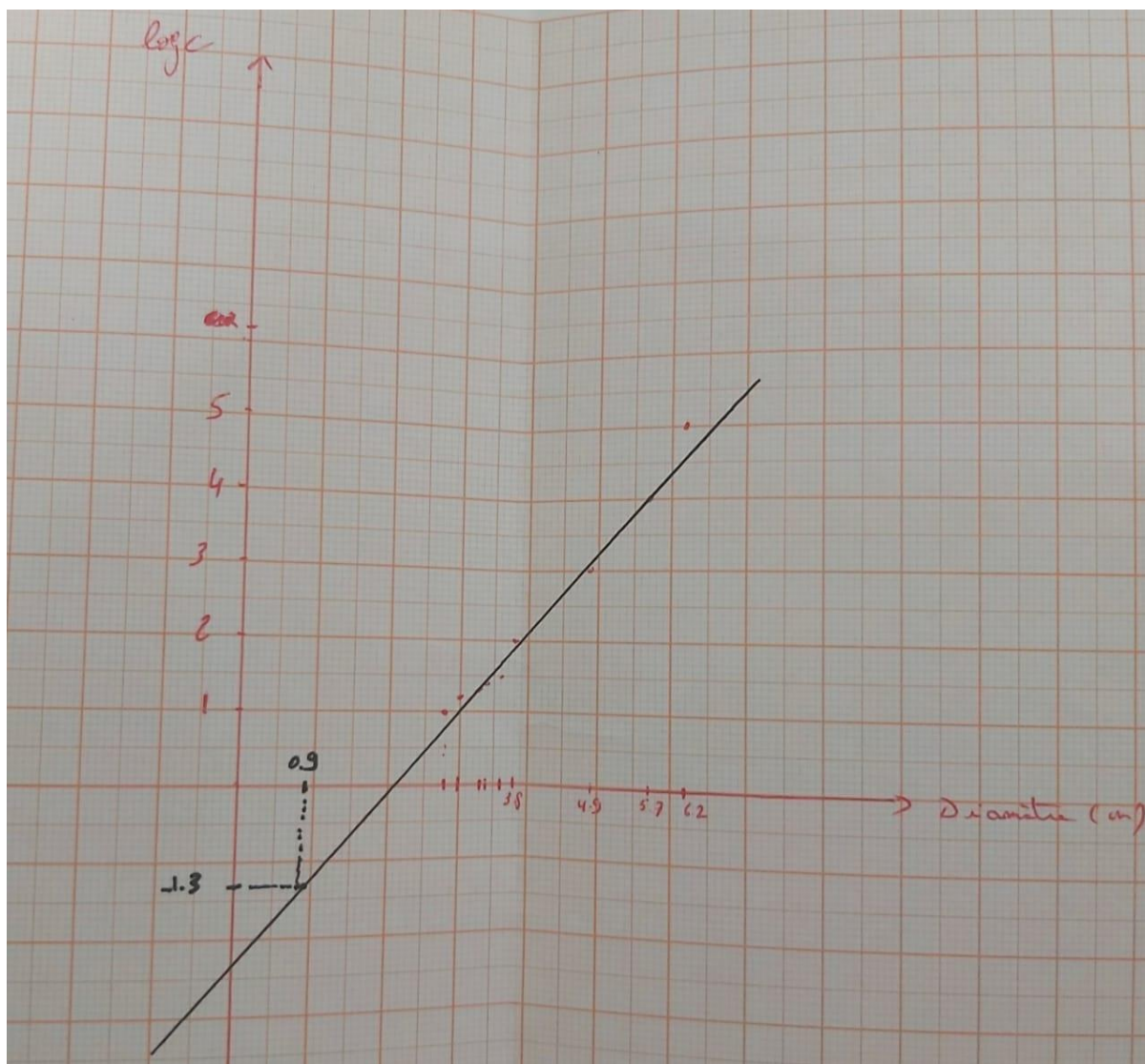
Diameter(cm)	2.8	3	3.3	3.4	3.6	3.8	4.9	5.7	6.2	0.9
LogC	1	1.2	1.3	1.39	1.47	2	3	4	5	?
Concentration(mg/ml)	10	16	20	25	30	102	103	104	105	



Graph 1: Graph showing the variation of penicillin concentration (LogC) as function of the diameter of the inhibition zone



References:



According to the calibration curve $y=1.15x+(-2.43)$

So the concentration of PenG in F1 is 0.04mg/ml

Quantification for Crude PenG obtained from F3 crystallization

Table 2 : Measurements of diameters of bacterial growth inhibition zones for different concentrations of standard dilute commercial penicillinG and F3 solution.

Diameter(cm)	3.8	3.5	3.4	3.2	3.1	0.7
--------------	-----	-----	-----	-----	-----	-----

LogC

1.47

1.39

1.30

1.20

1

?

Concentration(mg/ml)

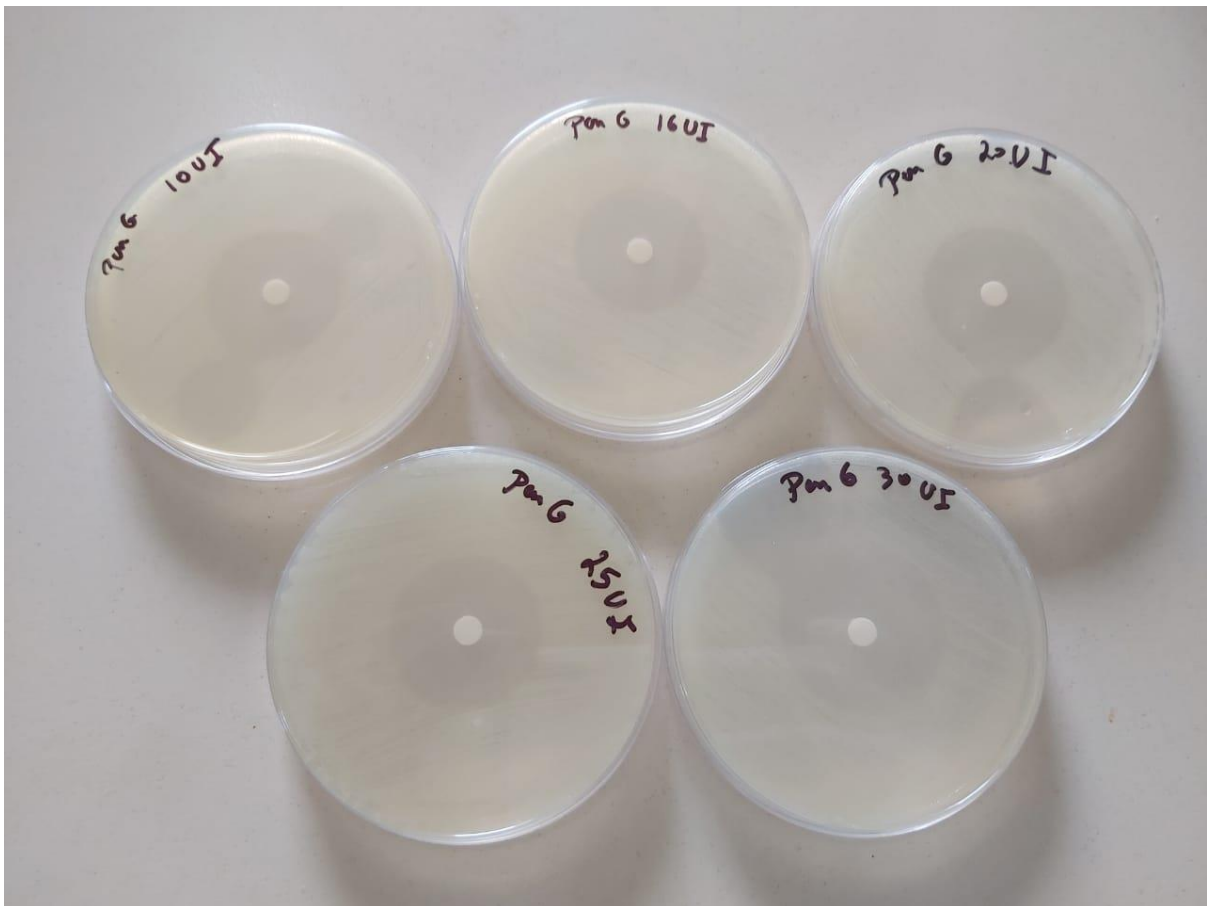
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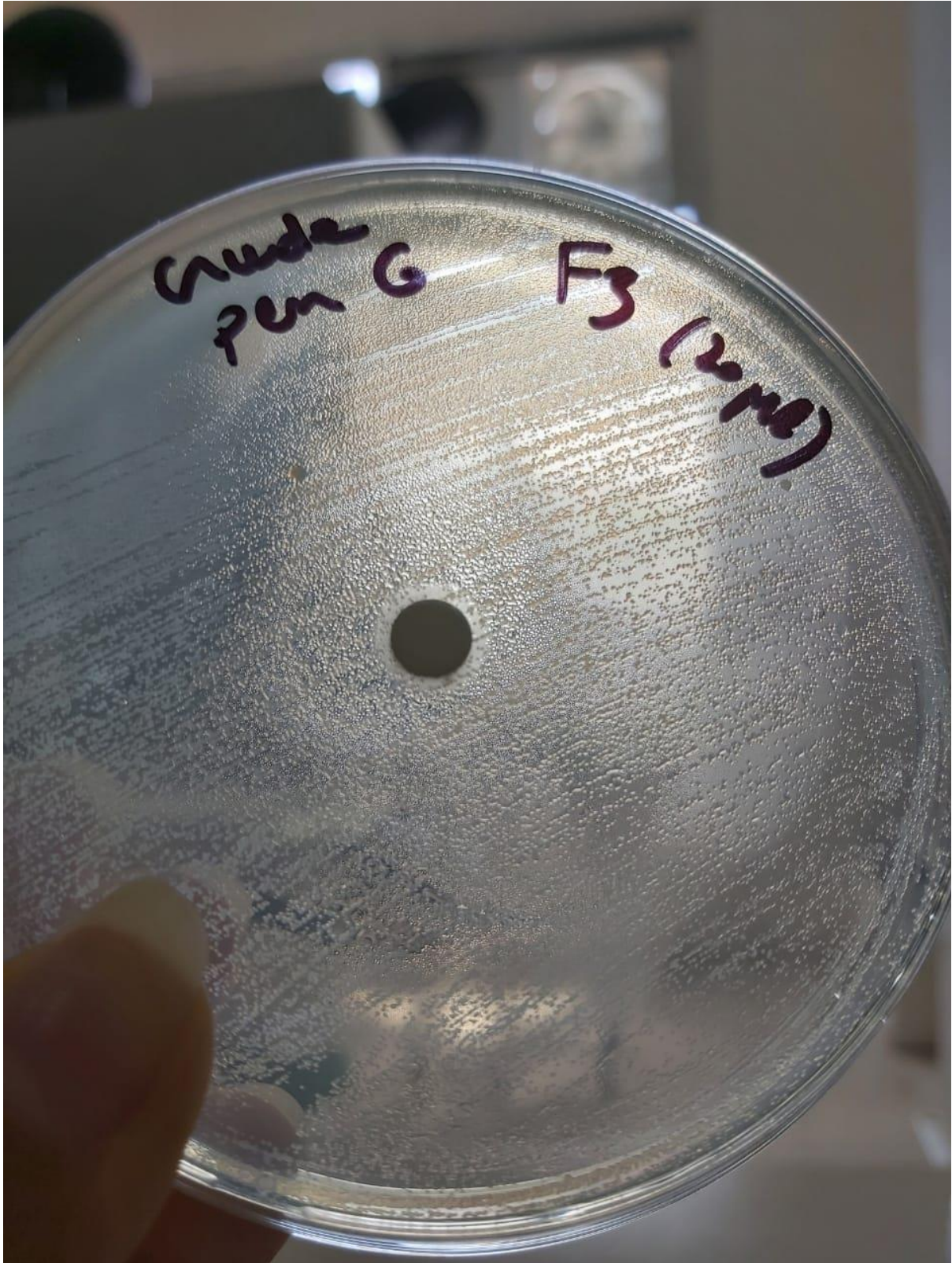
20

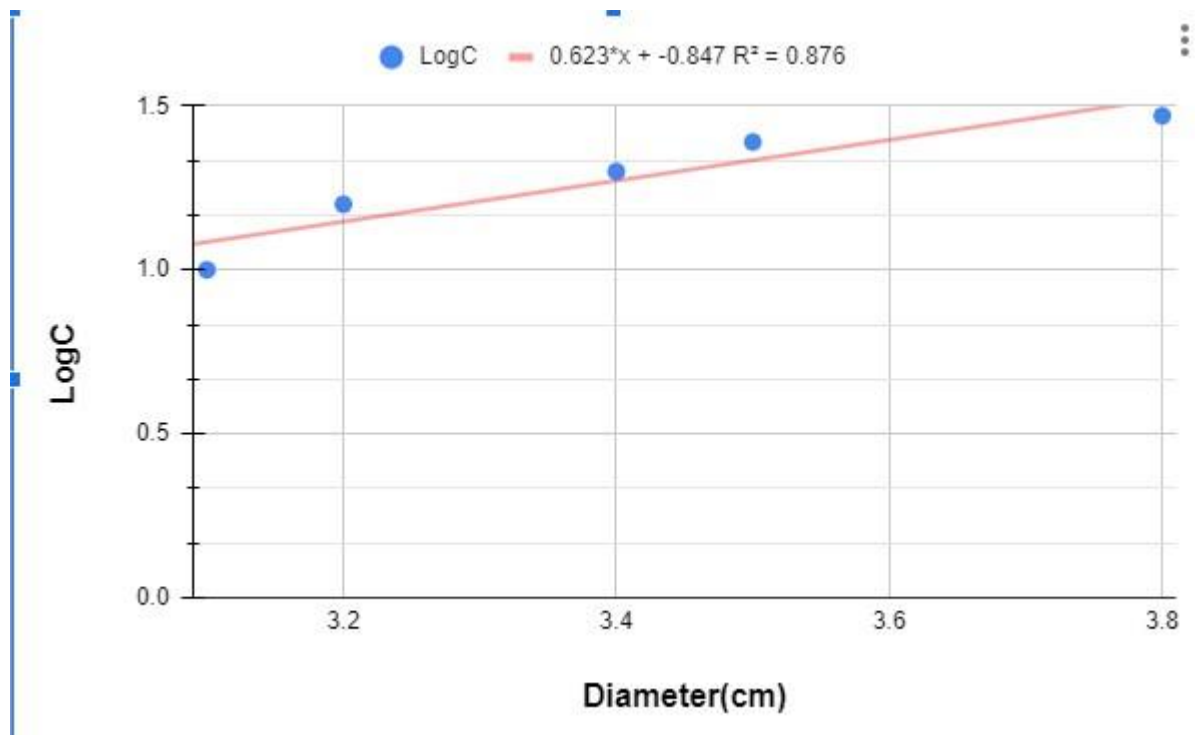
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10



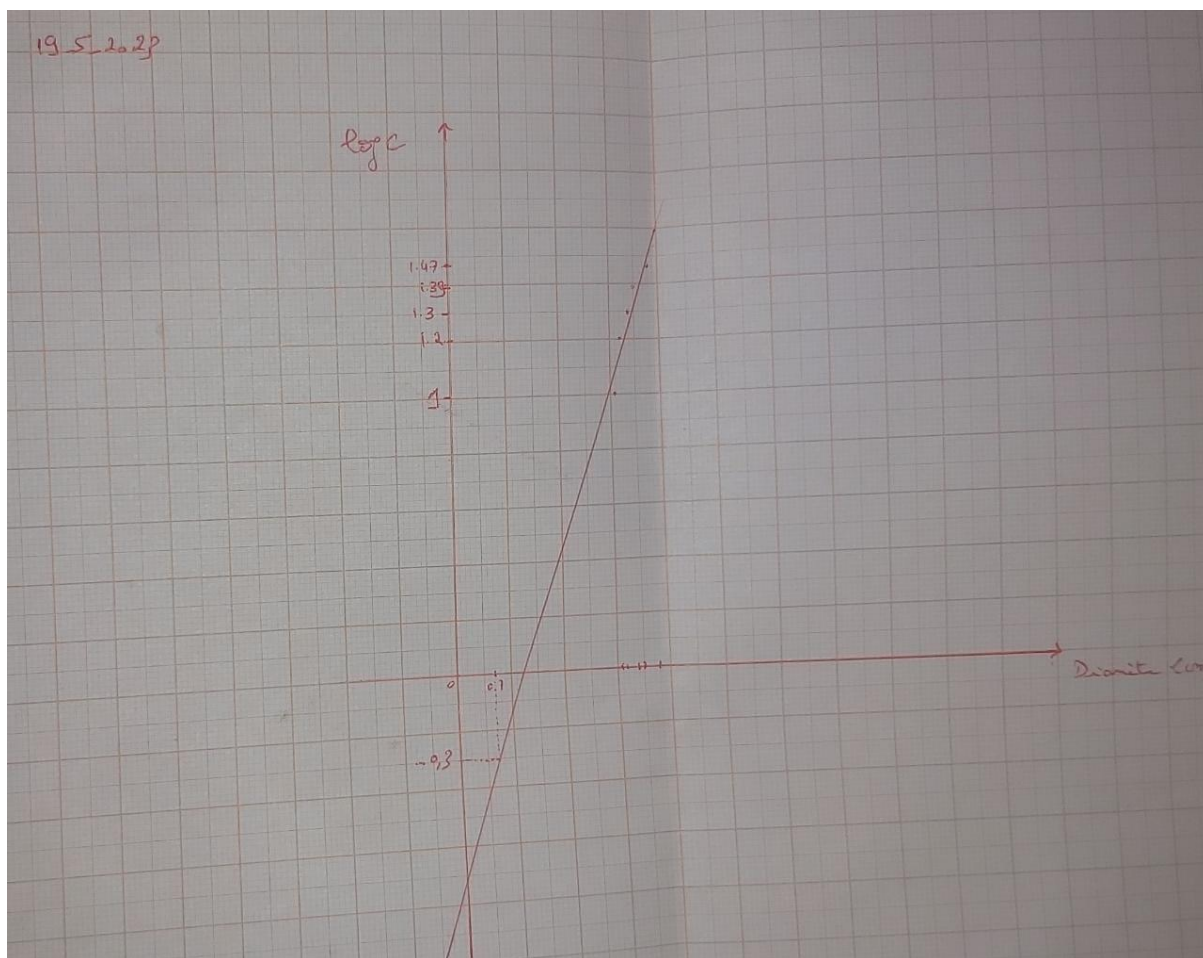
References:





Graph 2 : Graph showing the variation of penicillin concentration (LogC) as function of the diameter of the inhibition zone.

References:



According to the calibration curve $y=0.623x+(-0.847)$

So the concentration of PenG in F3 is 0.388mg/ml ~ 0.4 mg/ml.

Table showing every step with its duration and working date

Steps of preparation	Working Date	Duration(Days)
Penicillium culture	April 19	4 days
Fermentation	April 24 to May 3	10 days
Purification: filtration	May 4 to May 5	2 days
Crystallization	May 5 to May 12	7 days

Sensitivity test	May15 to May16	2 days
Quantification/results F1	May15 to May16	2 days
Sensitivity test	May18 to May 19	2 days
Quantification/result F3		

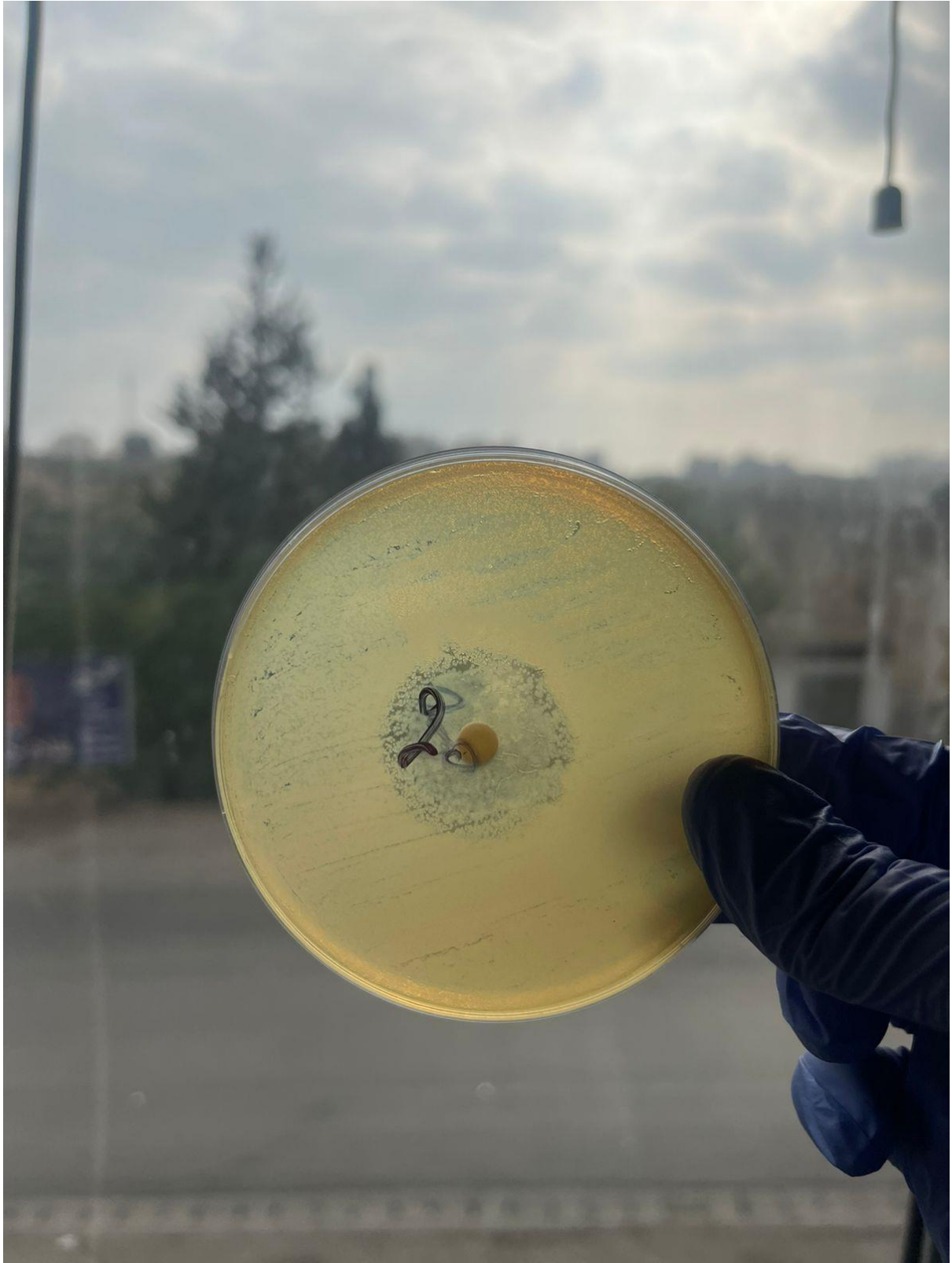
Trial 3:

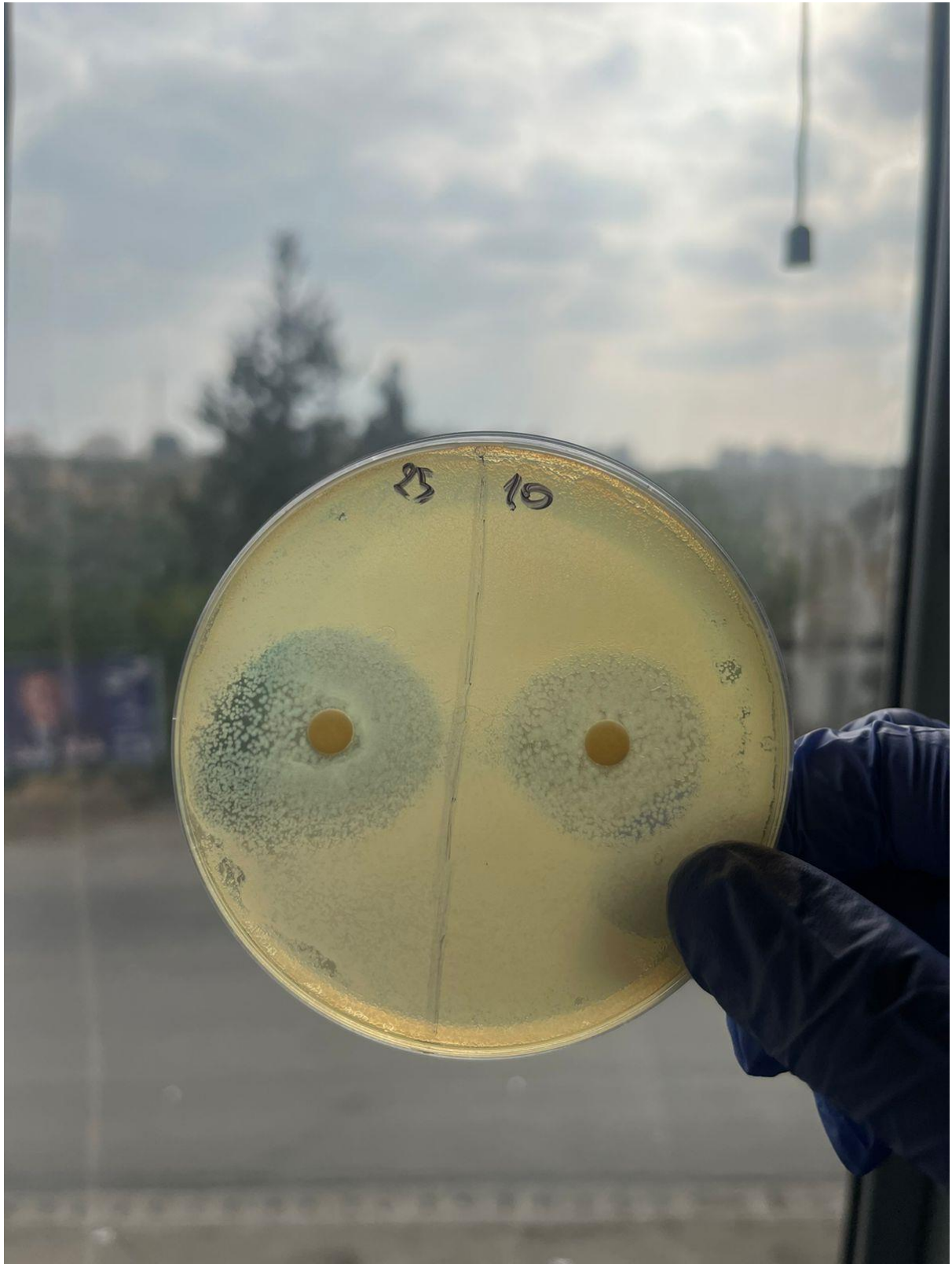
Same protocol reused in this trial (except: adding PAA : Phenylacetic acid every 24h at 30°C for the first 48h then decrease the temperature to 25°C for the rest of the fermentation).

PS : due to disfunction of the incubator we couldn't maintain the temperature (between 25-30°C) and solubility of the PAA

RESULTS:

References:





References:



Diameter (cm)

2.6

2.6

2.7

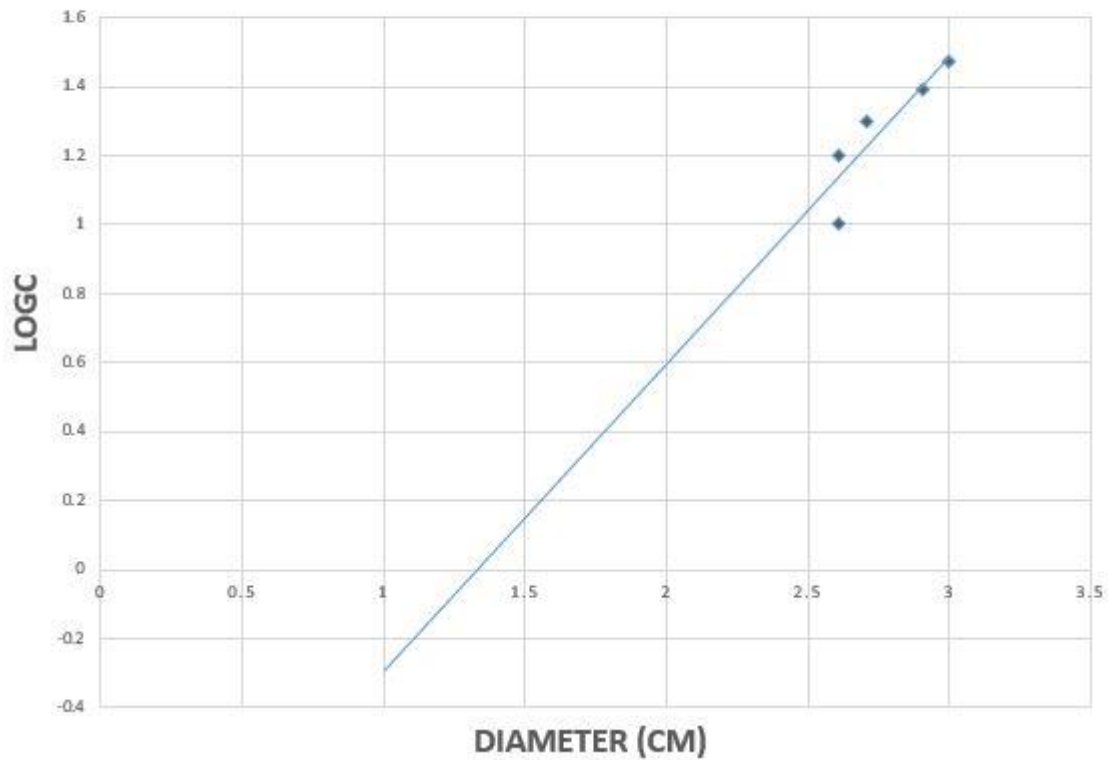
2.9

3

1

Log C	1	1.2	1.3	1.39	1.47	-0.3
Concentration (mg/ml)	10	16	20	25	30	0.5

GRAPH SHOWING THE VARIATION OF PENICILLIN CONCENTRATION (LOG C) AS FUNCTION OF THE DIAMETER OF THE INHIBITION ZONE.



[Word-docx-Stage-report-ghinwayounes-2023](#)

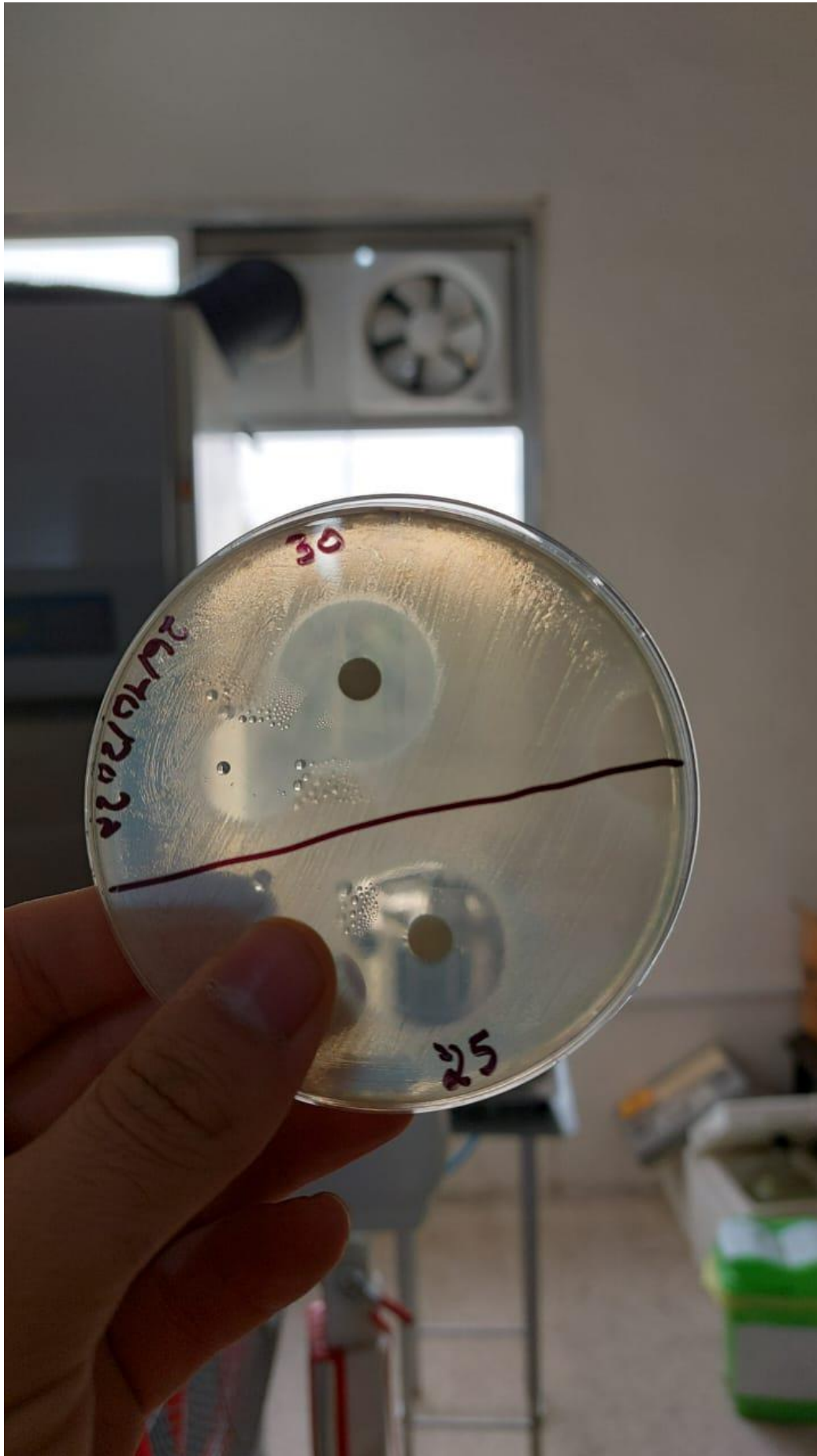


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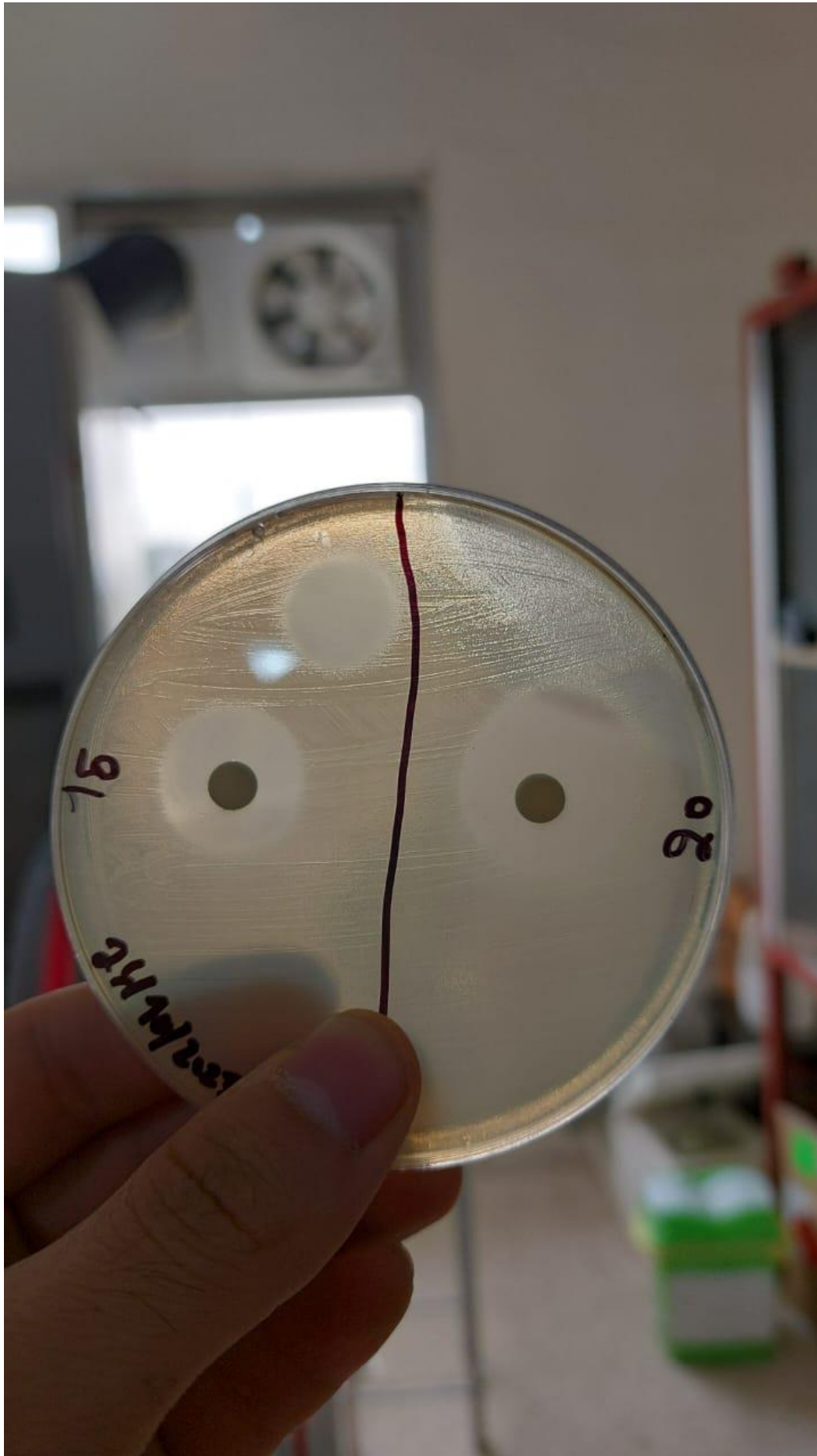
trial 4

Same protocol reused in this trial (except: aerobic respiration and medium: without glucose , lactose 3g).

results



References:

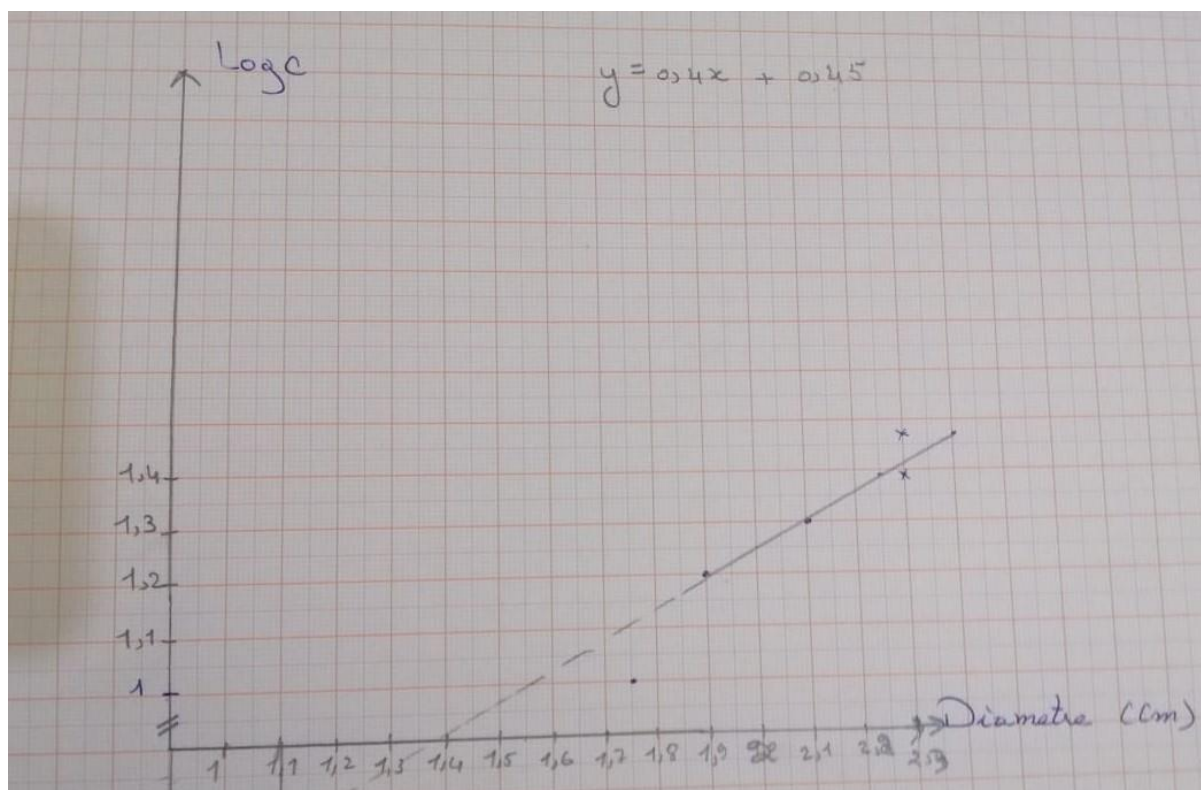


References:



References:

Diameter (cm)	2.4	2.2	2.1	1.9	1.75	1.1
Log c	1.47	1.39	1.30	1.20	1	0.89
Concentration(mg/ml)	30	25	20	16	10	7.76



Graph showing the variation of penicillin concentration (LogC) as function of the diameter of the inhibition zone.

4 Part 3: Ampicillin

4.1 Ampicillin Production and Quantification 2023

Ampicillin Synthesis Steps

Preparation of the solution of ampicillin synthesis from our crude produced penicillin:

- Measure a volume of 7.5 mL from our produced penicillin(reserved in PB solution), using a graduated cylinder
- Add 0.8g of penicillin acylase (PGA)
- Agitating for 1h
- Add 7.5ml of the ester D-(-)- α -Phenylglycine methyl ester hydrochloride, D-PGMEH (0.24g ester in 10 ml potassium phosphate buffer) to the mix
- Add 0.24g enzyme (PGA) again
- Adjust the PH for about 6.4-7 by adding NaOH
- Put the beaker containing the bar magnet on the magnetic stirrer for 22.5 hours

(Take 1ml of the filtrate for the bacterial sensibility test later A1)

References:



Ampicillin purification and harvesting:

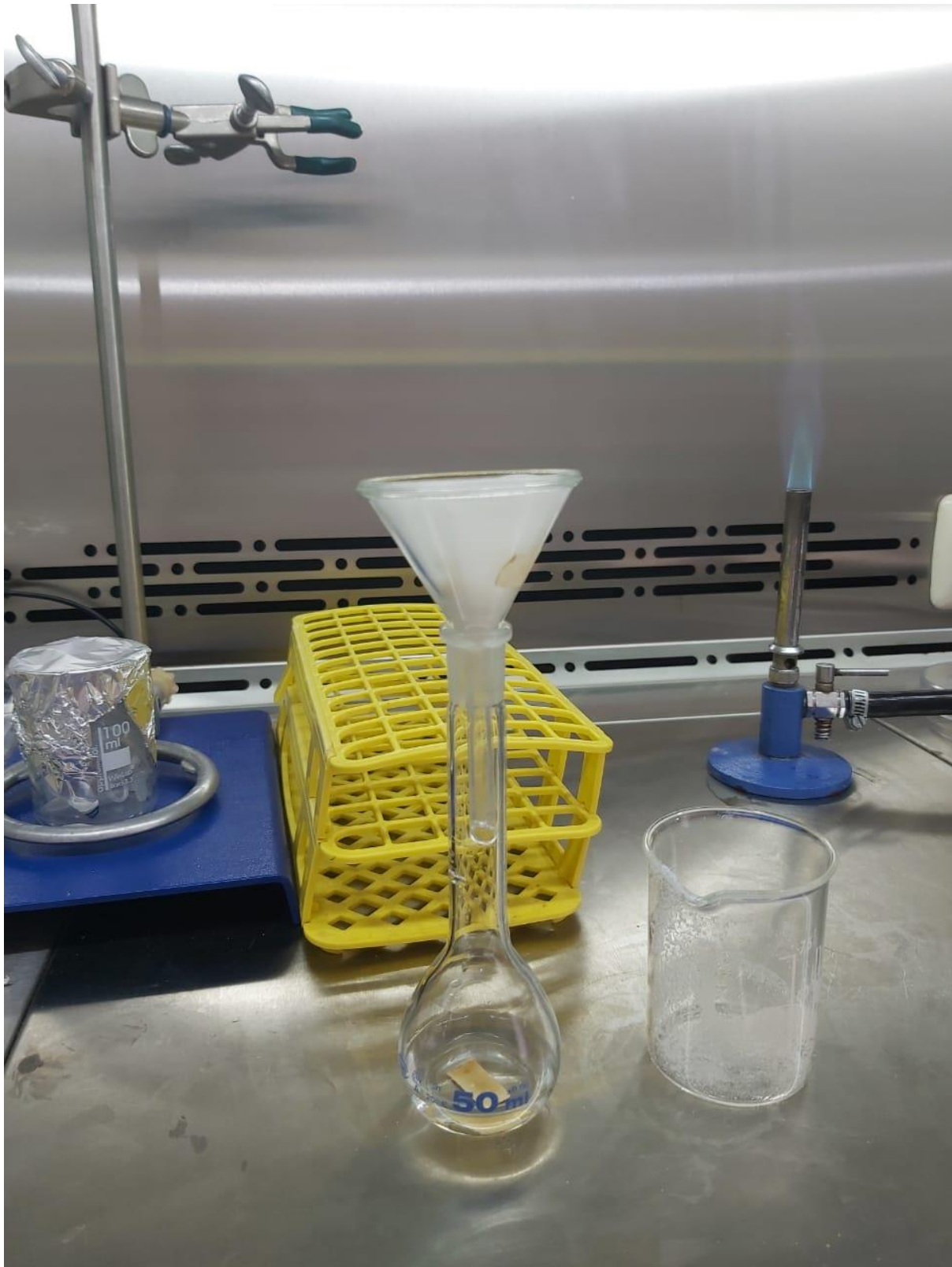
- Add few drops of H_2SO_4 to stop the enzymatic reaction and adjust the PH to 2
- Filtrate the mix using a funnel fitted with filter paper
- Add butyl acetate solvent (1Vsolvent/2Vfiltrate)(4ml/8ml)
- Let rest for 2 minutes, then the organic phase is removed and the aqueous phase is discarded after decantation

(Take 1ml from aqueous phase A2 and organic phase A3 to test the presence of ampicillin later)

- Add 2% phosphate buffer (V/V)(4ml/4ml) to the organic solution

(we obtain an aqueous phase B1 and an organic phase B2)

- Adjust the PH to 7.5 by adding NaOH



References:





Crystallization:

- Add 2% (W/V)(~0.1g/4.3ml) NaHCO_3 to the aqueous phase medium

References:

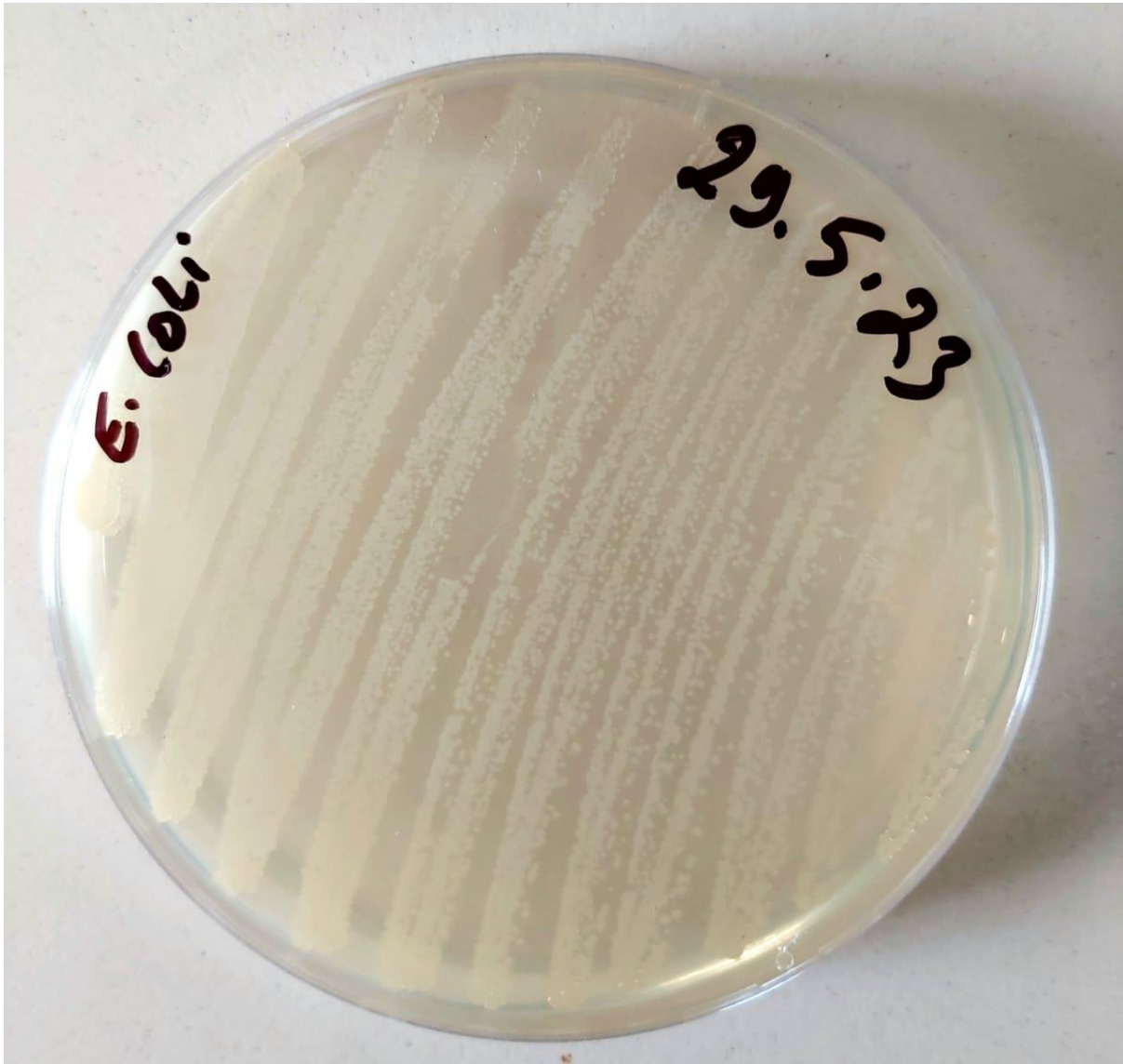
- Cool them at 4°C for about 7 days
- Filtrate them to harvest ampicillin sodium salt

Ampicillin Quantification (Standard Protocol):

- The one bacterial strain which can be used: E. coli
- Regeneration of Escherichia coli Bacteria

Place the isolated colony of E. coli on a new standard agar petri dish and then spread it

Incubate the bacteria for approximately 18 hours in an incubator at 37°C



- Qualitative antibiogram, using disc diffusion method

To test the effectiveness of the antibiotic on the bacteria

- Take 3 to 5 colonies of the isolated colonies of E. coli with a loop
- Add them in 2ml sterile saline (NaCl 0.9%)
- Vortex the saline tube to create a smooth suspension
- Adjust the turbidity of this suspension to a 0.5 McFarland standard by adding more organism if the suspension is too light or diluting with sterile saline if the suspension is too heavy.
- Inoculate the surface of Mueller Hinton agar plate by streaking the swab 3 times over the entire agar surface
- Sit the plate at room temperature at least 3 to 5 minutes (but no more than 15 minutes) to let the surface of the agar plate dry before proceeding to the next step
- Reverse the plate and divide it into 6
- Deposit 5 disc in each quadrant with an empty quadrant used as control negative
- Add 20µl of each sample on a disc
- Reverse the plate and incubate it at 37°C for 18 to 24 h

Results and Discussion

The sensitivity test shows an important zone of inhibition in A3 and B2 proving the presence of crude produced ampicillin obtained from our crude penicillin so the reaction has succeeded.

The test was validated while repeated twice.

Samples was taken from:

A1: solution obtained after the first filtration before adding the organic solvent

A2: Aqueous phase after butyl acetate extraction

A3: Organic phase after butyl acetate extraction

B1: Aqueous phase after PB extraction

B2: Organic phase after PB extraction

References:

The results show :

- no zone of inhibition in A1 (sample taken before adjusting the 2)
- No zone of inhibition in the aqueous phase A2 so the total produced ampicillin is extracted into the organic phase A3
- A small zone of inhibition in A3 so there is a loss while extracting ampicillin from this phase
- No zone of inhibition in the Aqueous phase B1 with an important one in the organic phase B2 so PB isn't the right solvent for ampicillin extraction for crystallization.



Crude Ampicillin quantification

Preparation for commercial ampicillin quantification

References:

While the commercial ampicillin used in quantification had no results on E.coli

We decided to use amoxicillin instead since it has an identical antibacterial activity with ampicillin.

Crude Ampicillin quantification

Preparation for commercial ampicillin quantification

While the commercial ampicillin used in quantification had no results on E.coli

We decided to use amoxicillin instead since it has an identical antibacterial activity with ampicillin.



- Weigh 1g of commercial amoxicillin
- Add 5 ml of potassium phosphate buffer to the amoxicillin
- Filtrate the mix using a sterile funnel and filter paper
- Store the mix (C standard amoxicillin=200 mg/ml) in the fridge for later use
- Prepare the sterile tubes with different concentration 20, 15, 12, 10, 8 et 5 mg/mL

TUBES	Solution ml	Distilled water ml
-------	-------------	--------------------

References:

Tube1 20mg/ml	1	9
Tube2 15mg/ml	7.5	2.5
Tube3 12mg/ml	6	1.5
Tube4 10mg/ml	5	1
Tube5 8mg/ml	4	1
Tube6 5mg/ml	2.5	1.5

Quantification of the produced ampicillin using the disc diffusion method:

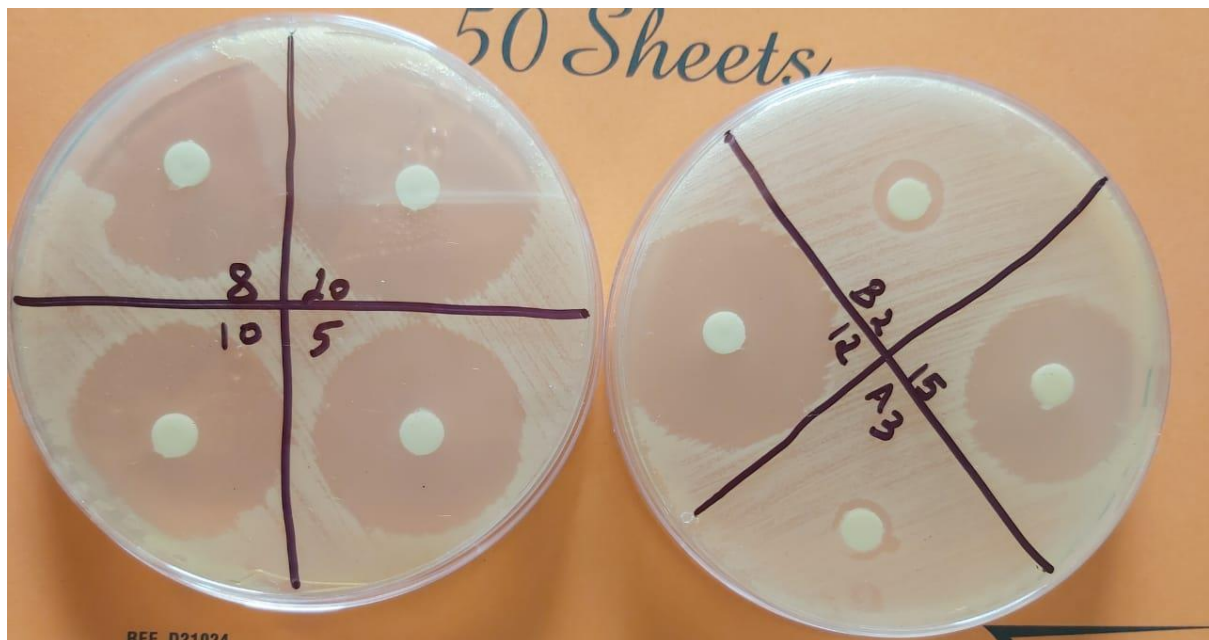
- Take 3 to 5 colonies of the isolated colonies with a loop, and we added them in 2ml sterile saline (NaCl 0.9%)
- Vortex the saline tube to create a smooth suspension.
- Adjust the turbidity of this suspension to a 0.5 McFarland standard(annex)
 - by adding more organism if the suspension is too light or diluting with
 - sterile saline if the suspension is too heavy.
- Use this suspension within 15 minutes of preparation.
- Inoculate the surface of 3 Mueller Hinton agar plate by streaking the swab 3 times over the entire agar surface, we rotated the plate approximately 60° each time to ensure an even distribution of the inoculum (use a control plate with E. coli on mueller Hinton agar)
- Leave the plates at room temperature at least 3 to 5 minutes (but no more than 15 minutes) for the surface of the agar plate to dry before proceeding to the next step.
- Reverse the plates and divide it into 4
- Deposit a disc in each quadrant

- Add 20µl of each concentration on a disc with the unknown one (A3 and B2)
- Reverse the plate and incubate it at at 37o for 18 to 24 h
- After the growth time, measure the zone of inhibition that had appeared using a ruler.
- Draw a graph showing the concentration of amoxicillin as a function of the diameter in order to be able to quantify the produced ampicillin (diameter as a function of Log C).

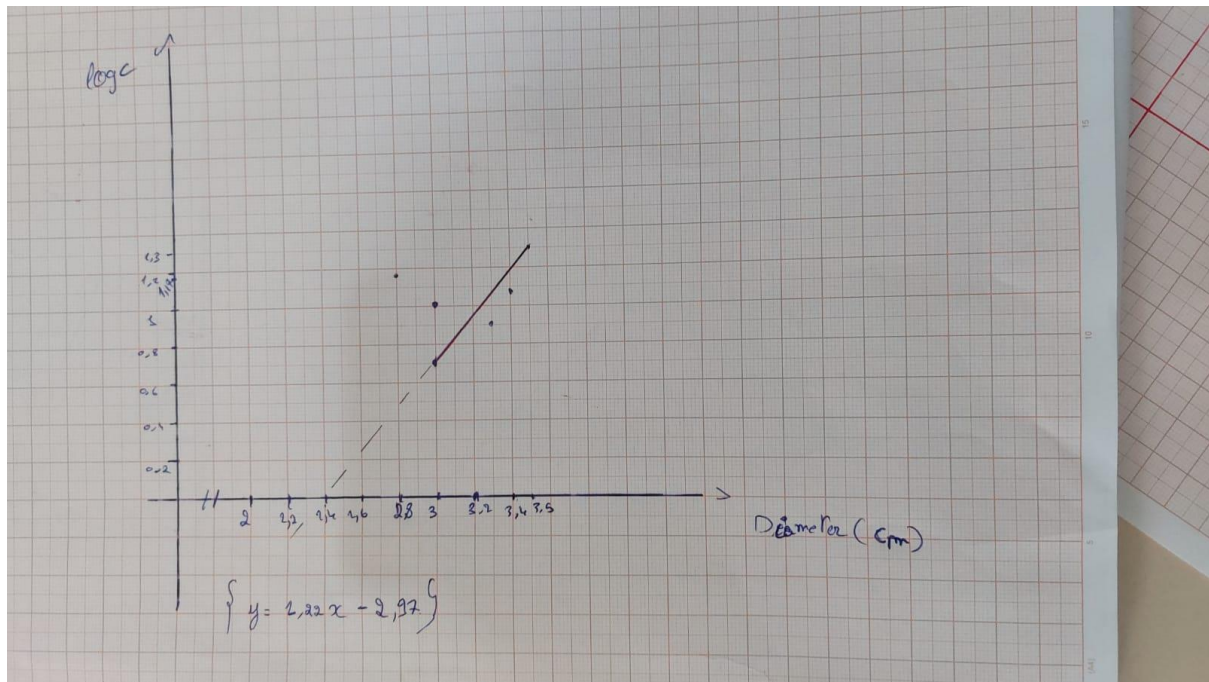
Results

Table 1 : Measurements of diameters of bacterial growth inhibition zones for different concentrations of standard dilute commercial amoxicillin and A3,B2.

Diameter(cm)	6	2.7	3.5	3.2	3.4	3.1	1(A3)	1.1(B2)
LogC	1.30	1.17	1.07	1	0.90	0.69	?	?
Concentration(mg/ml)	20	15	12	10	8	5	?	?



References:



Graph 1 : Graph showing the variation of amoxicillin concentration (LogC) as function of the diameter of the inhibition zone.

According to the calibration curve $y = 1.22x - 2.97$

So for an inhibition zone of 1 cm the concentration is 0.01 mg/ml

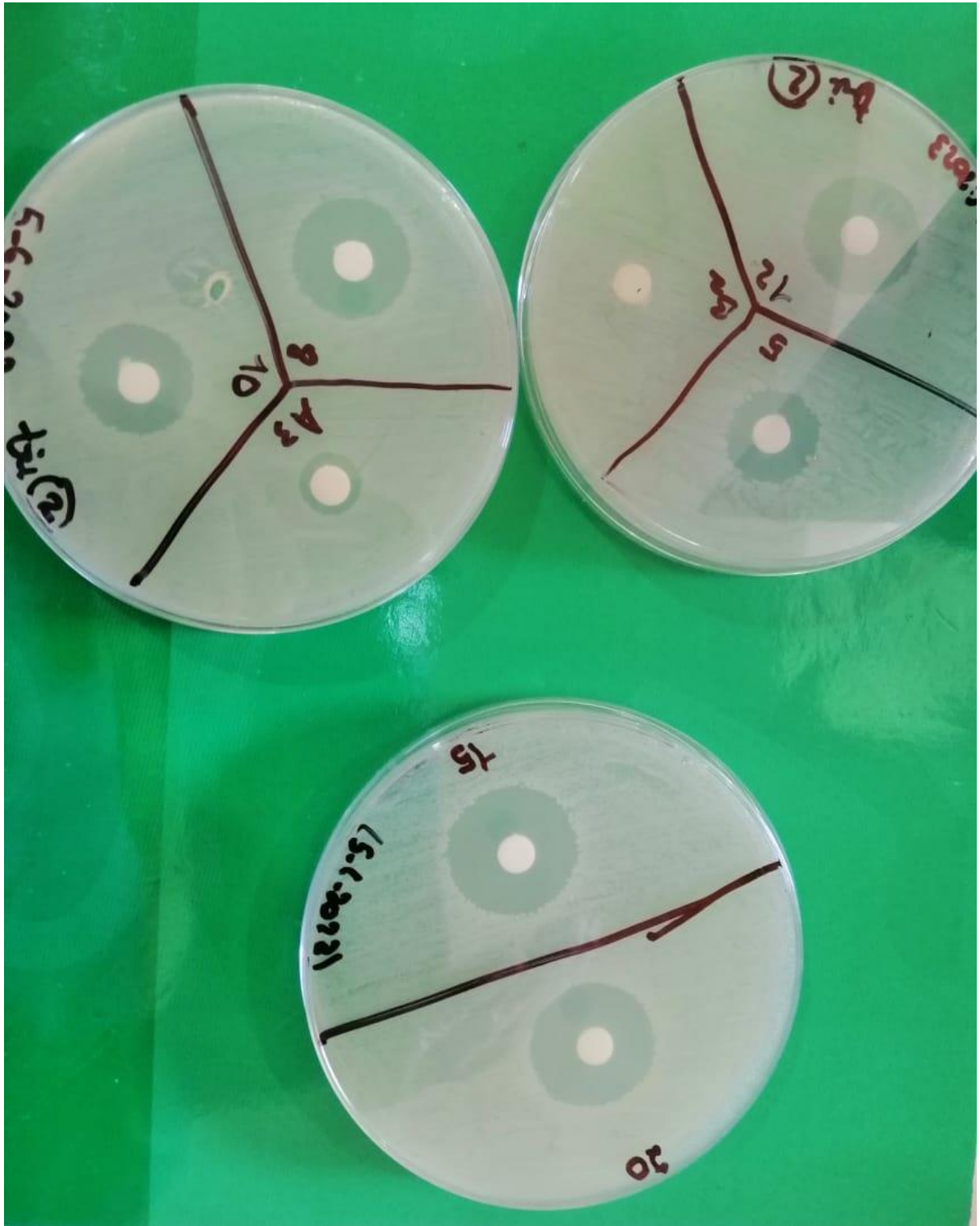
For an inhibition zone of 1.1 cm the concentration is 0.02 mg/ml

Results :

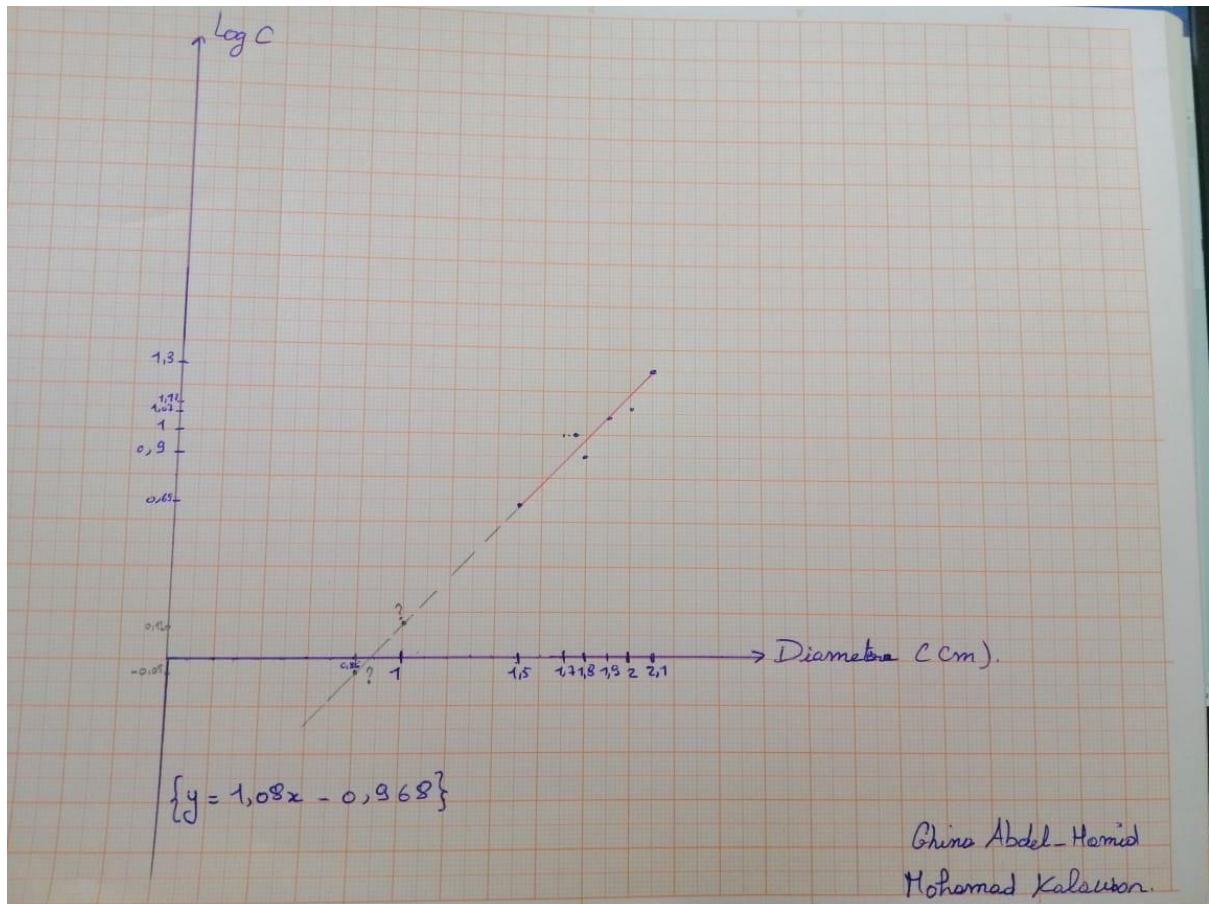
Trial 2 (6/6/2023)

Table 2: Measurements of diameters of bacterial growth inhibition zones for different concentrations of standard dilute commercial amoxicillin and A3,B2.

Diameter(cm)	2.1	2	1.9	1.75	1.8	1.55	1(A3)	0.85(B2)
LogC	1.30	1.17	1.07	1	0.90	0.69	?	?
Concentration(mg/ml)	20	15	12	10	8	5	?	?



References:



Graph 2 : Graph showing the variation of amoxicillin concentration (LogC) as function of the diameter of the inhibition zone.

According to the calibration curve $y=1.08x-0.968$

So for an inhibition zone of 1 cm the concentration is 1.31 mg/ml

For an inhibition zone of 0.85 cm the concentration is 0.89 mg/ml

Note: -We use the commercial amoxicillin instead ampicillin

-We filtrated the commercial amoxicillin

-Uncompleted inhibition in B2

-We should do a double number of Petri dish in target to reduce the error.

5 Part 4: Pilot plant scale drug production:

5.1 Mechanical design

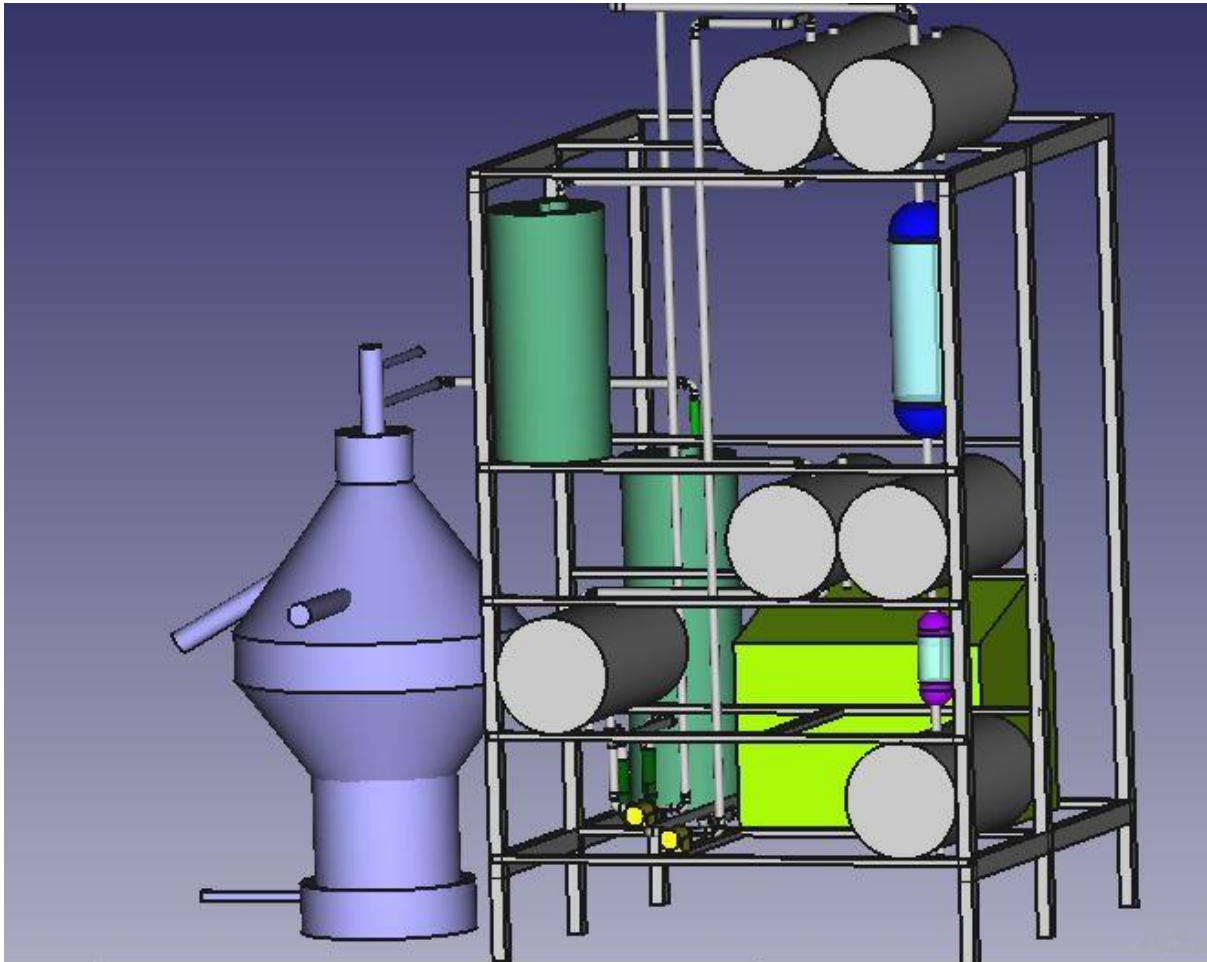


Image MEGBI penicillin/ampicillin/asprin pilot scale production old design

[FreeCAD MEGBI pen/amp/asp pilot scale version 2015](#)



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sembly_simplified_ma

References:

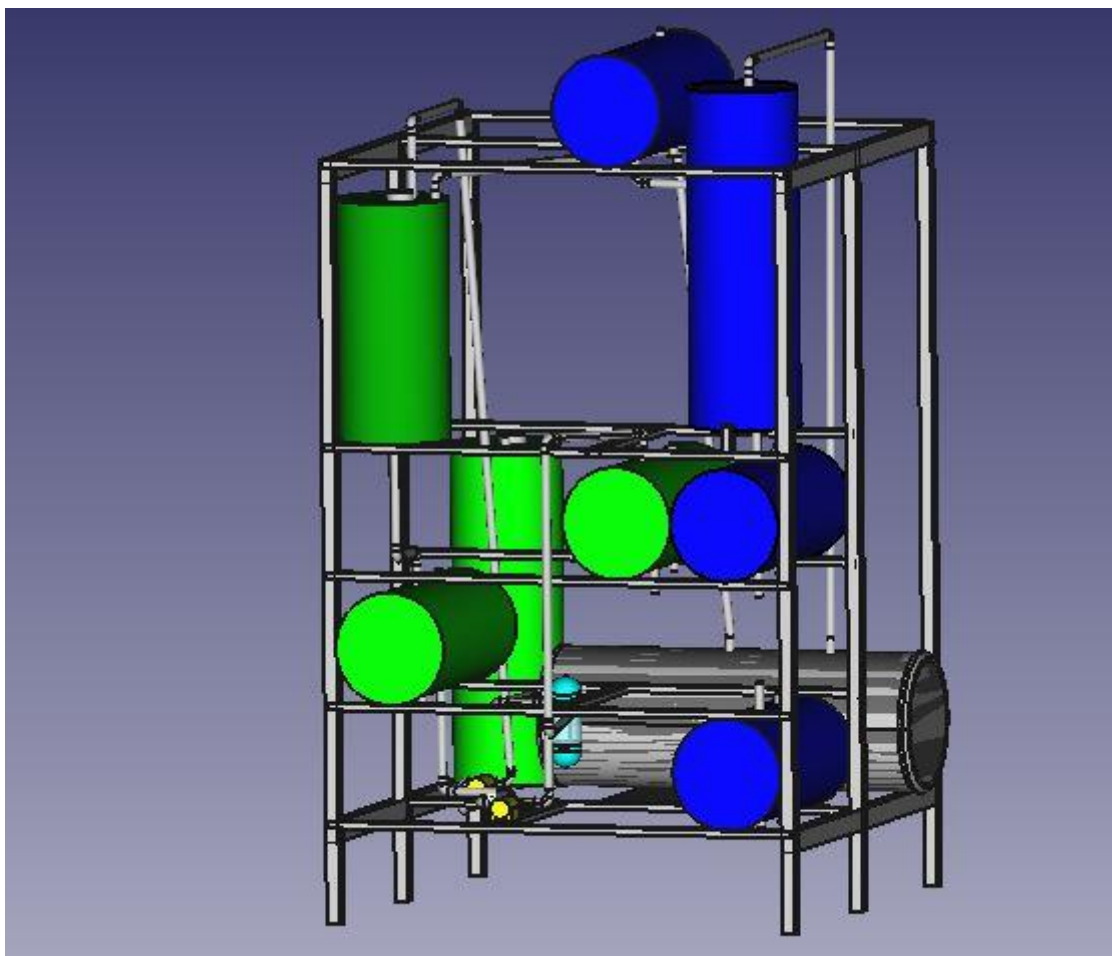


Image MEGBI penicillin/ampicillin/aspirin pilot scale production FreeCAD design
28/11/2022

PS:

Blue tanks : Aspirin

Green tanks : Penicillin/Ampicillin

[FreeCAD design MEGBI Pen/Amp/Asp pilot scale version 28/11/2022](#)



FreeCAD Pilot Scale
New design.FCStd

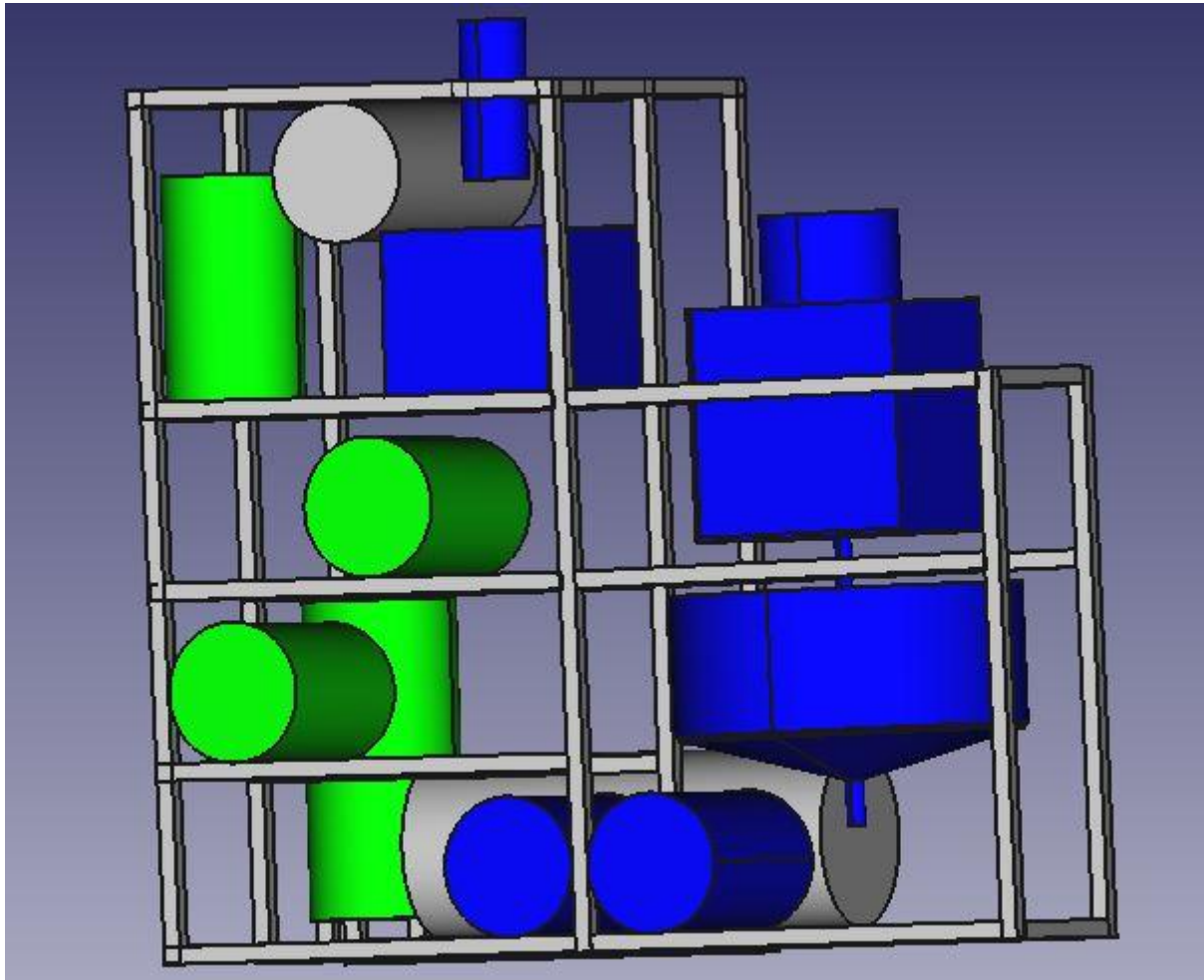


Image MEGBI Penicillin/ampicillin/aspirin pilot scale production FreeCAD design
16/12/2022

Blue tanks : Aspirin

Green tanks : Penicillin/Ampicillin

FreeCAD design Pen/Amp/Asp pilot scale version 16/12/2022


aspirin
filtration.FCStd

References:

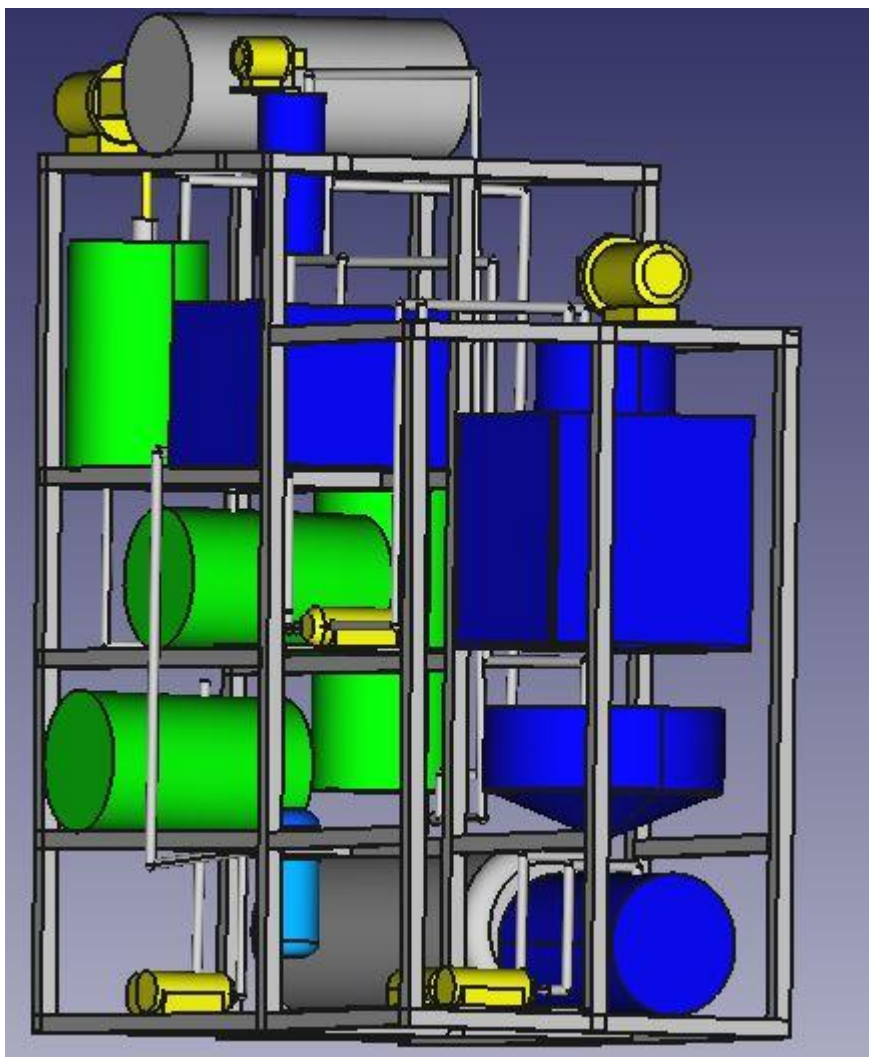


Image MEGBI Penicillin/Ampicillin/Aspirin pilot scale production FreeCAD design
23/01/2023 (achieved)

Blue tanks : Aspirin

Green tanks : Penicillin/Ampicillin

FreeCAD design Pen/Amp/Asp pilot scale version 23/01/2023



aspirin filtration new
design.FCStd

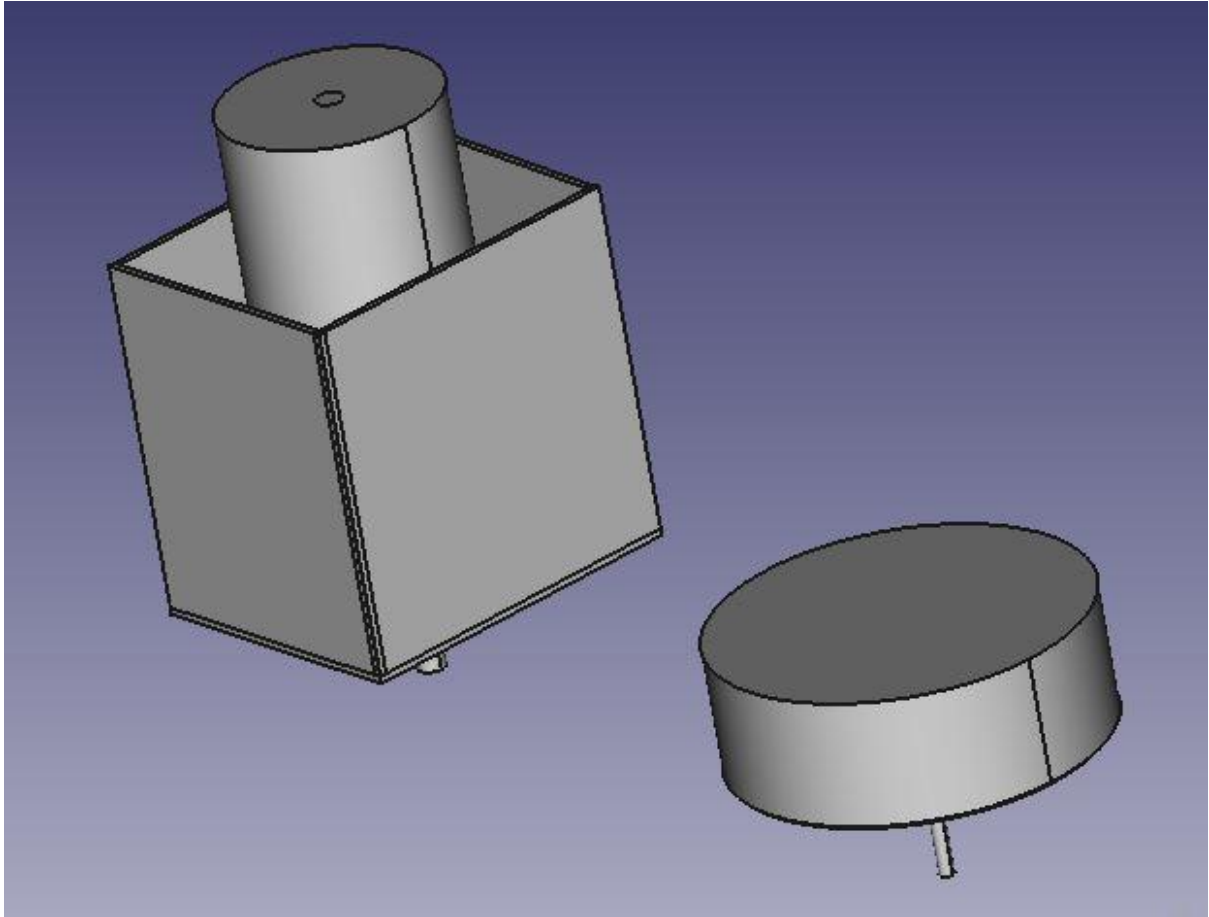


Image MEGBI Aspirin filter and crystallizer pilot scale FreeCAD design 09/01/2023.

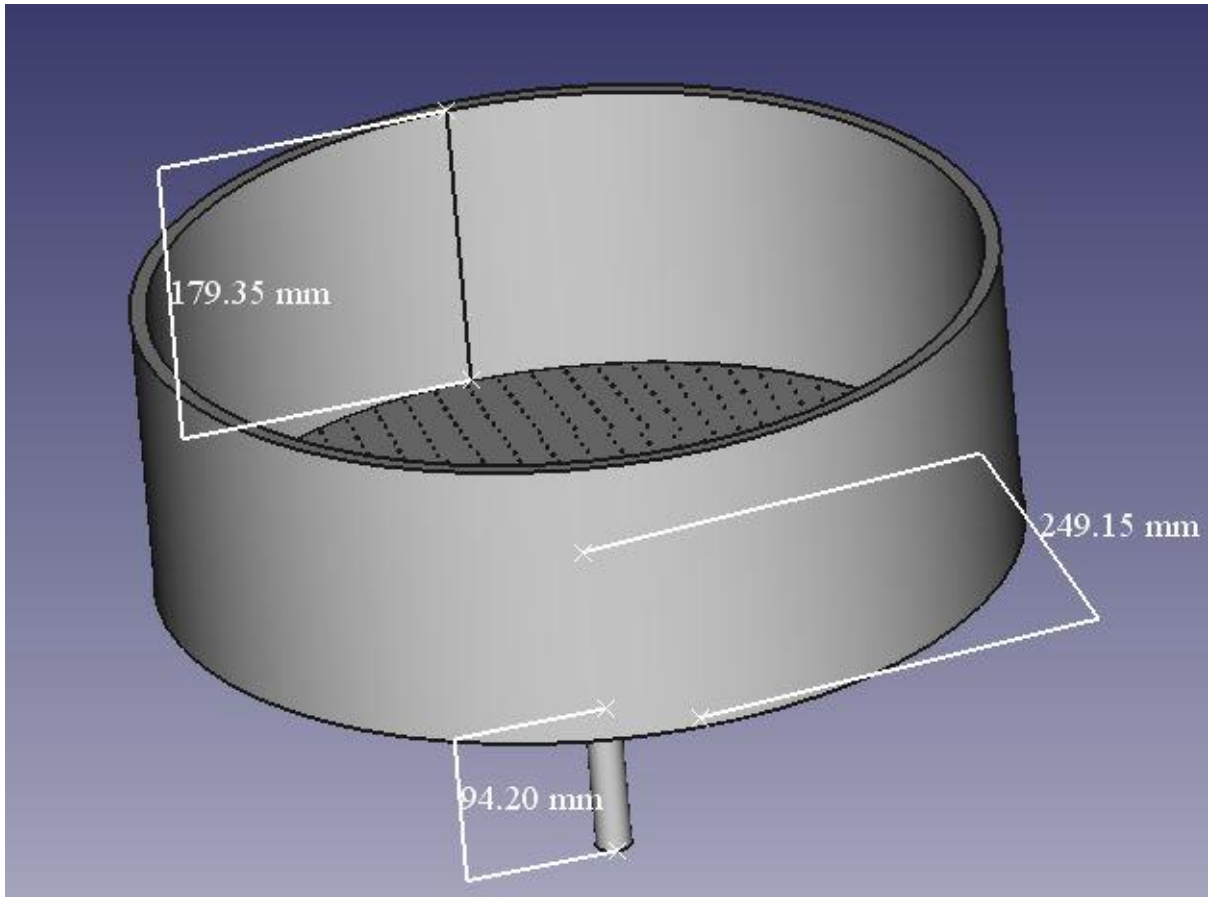
[FreeCAD design aspirin filter/crystallizer pilot scale version 10/01/2023](#)

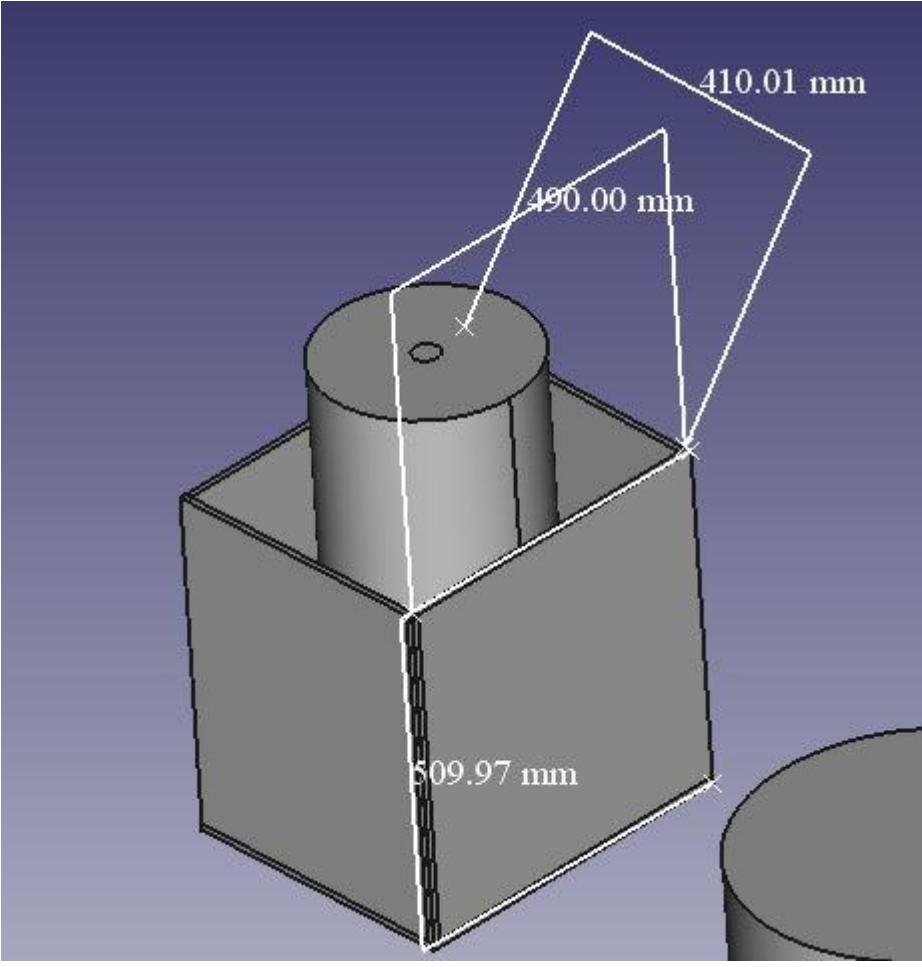


aspirin filter +
crystallizer.FCStd

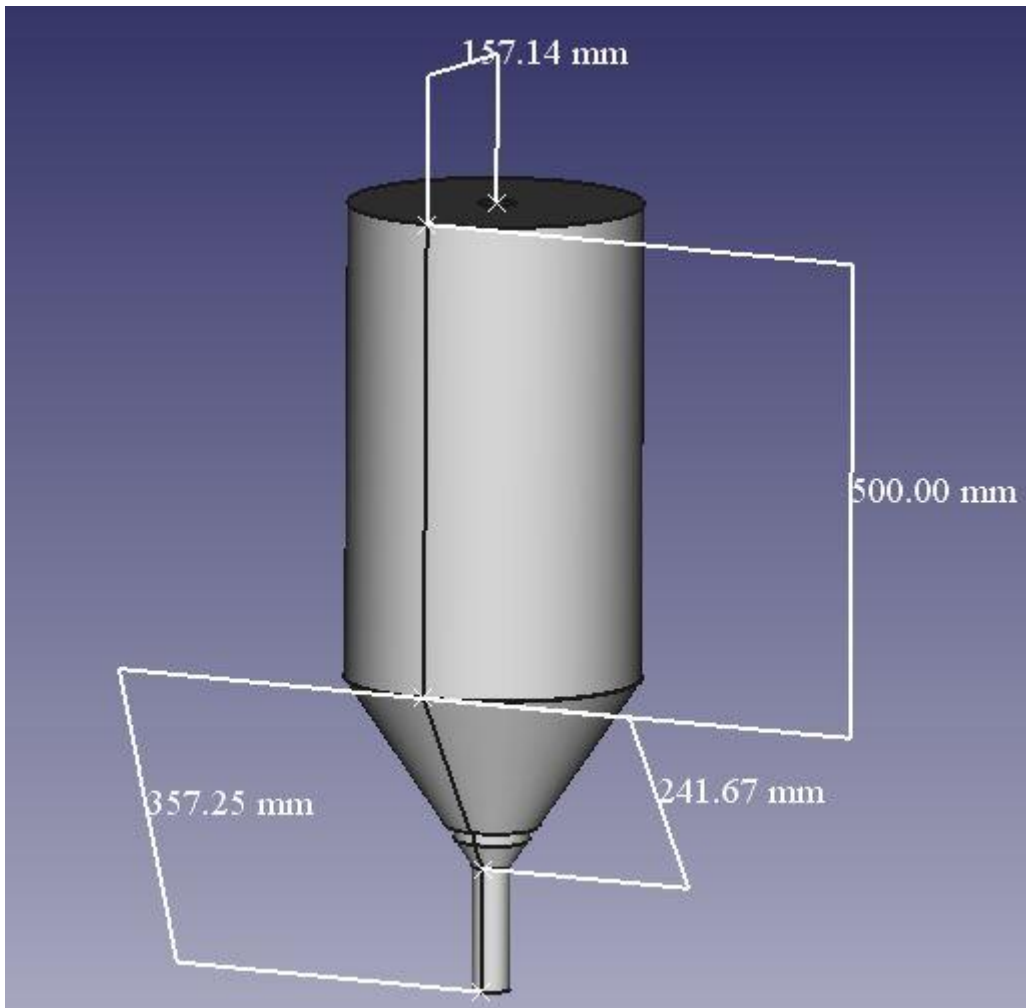
**Measurement and sizes of
filter/crystallizer aspirin pilot plan:**

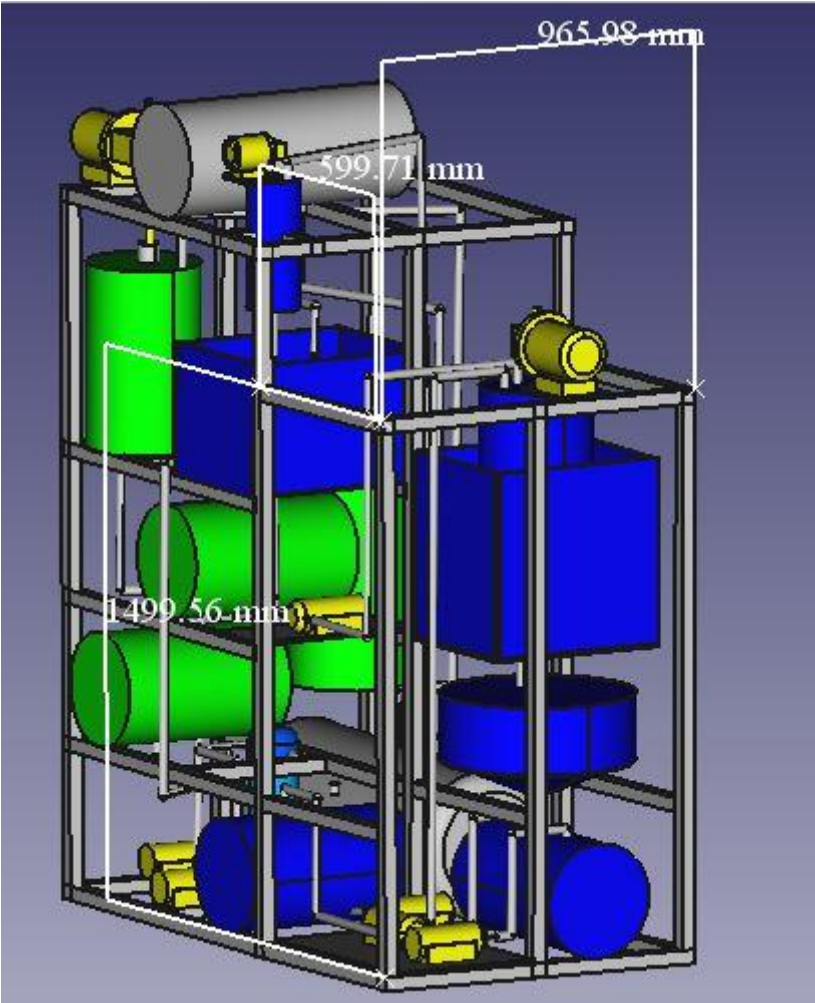
References:





References:





References:

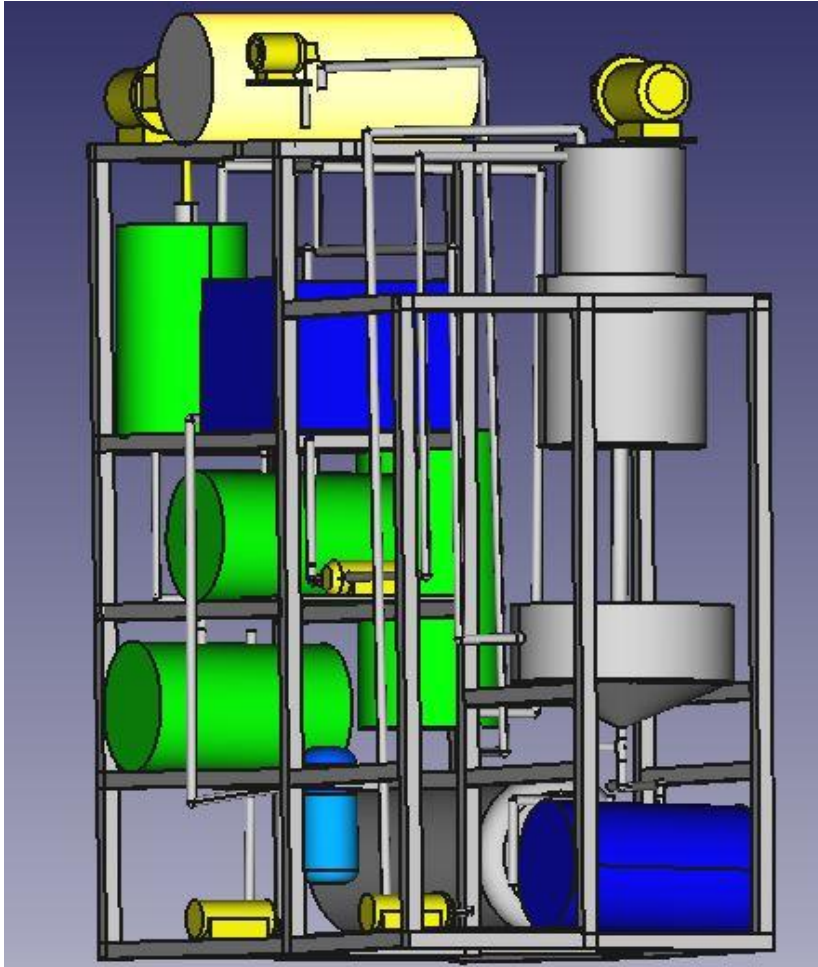


Image for lastet update for pilot plant ASPIRIN/PENICILLIN production 18/11/2023

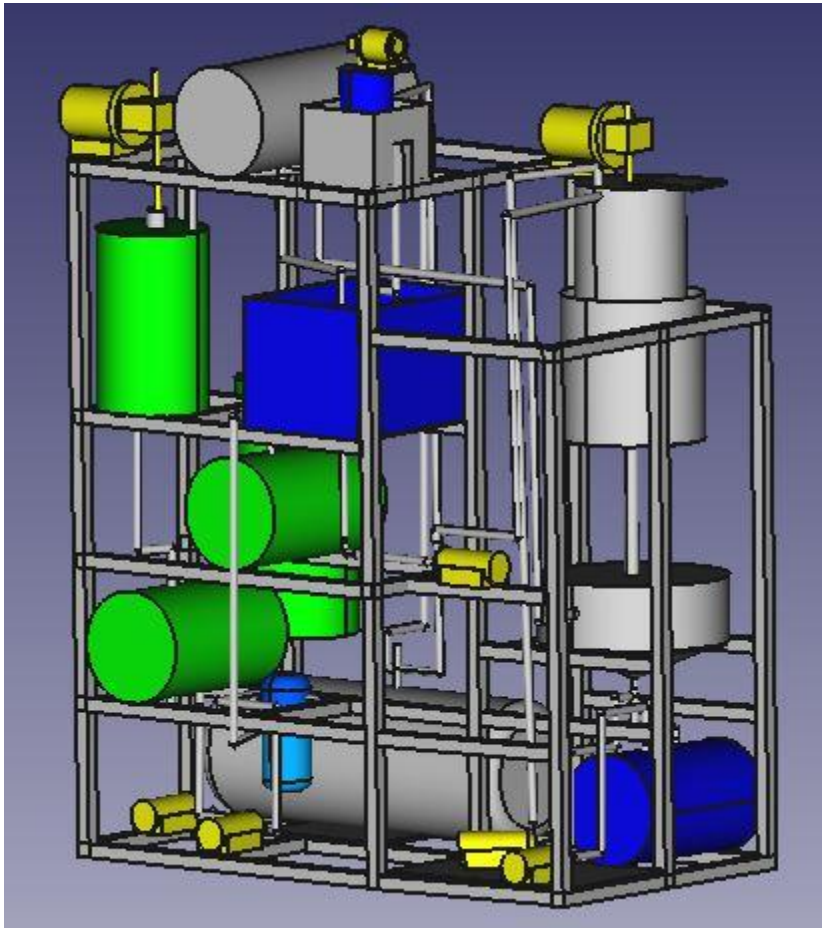
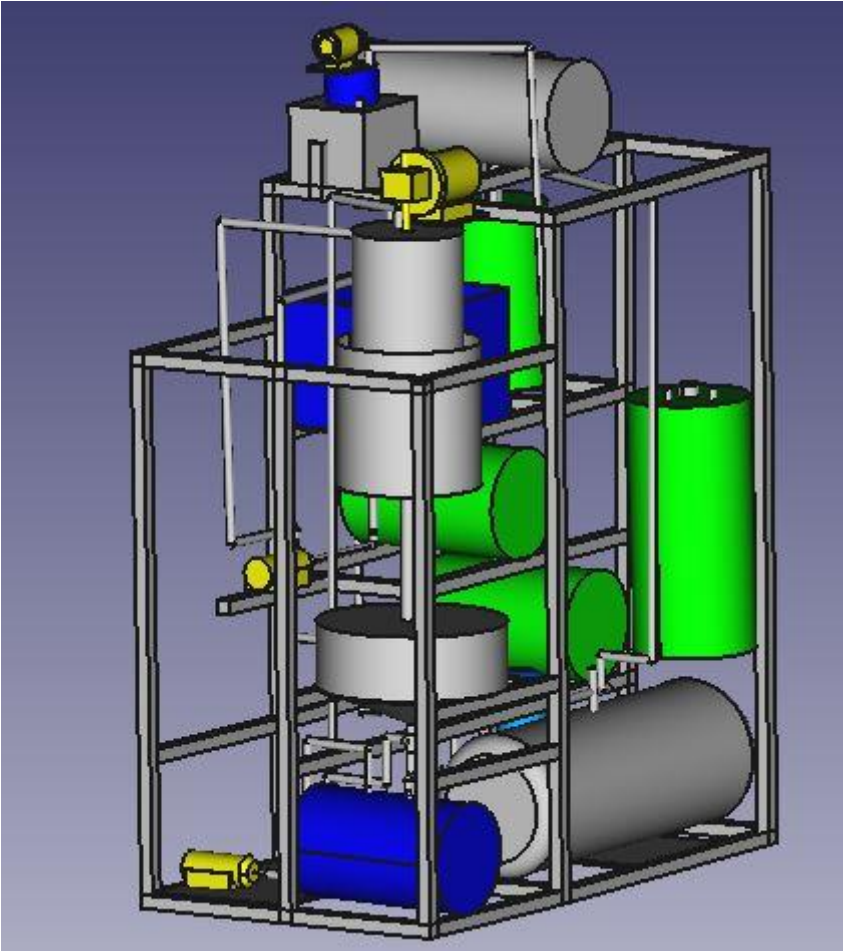


Image for lastet update for pilot plant ASPIRIN/PENICILLIN production 23/11/2023

References:

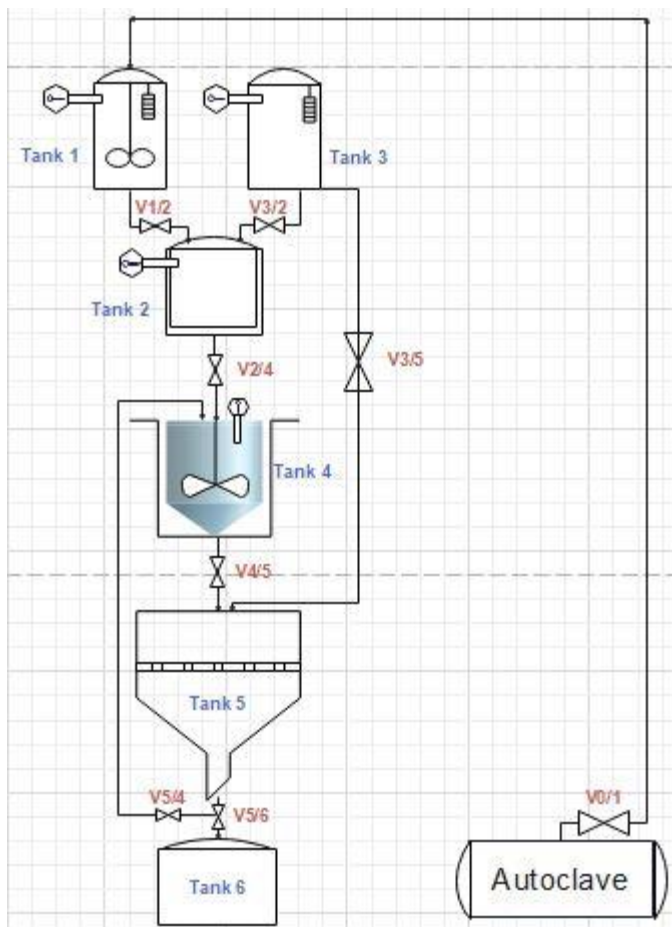


[FreeCAD design ASPIRIN/PENICILLIN Pilot plant 23/11/2023](#)



aspirin filtration new
design0.FCStd

5.2 pilot plant system design

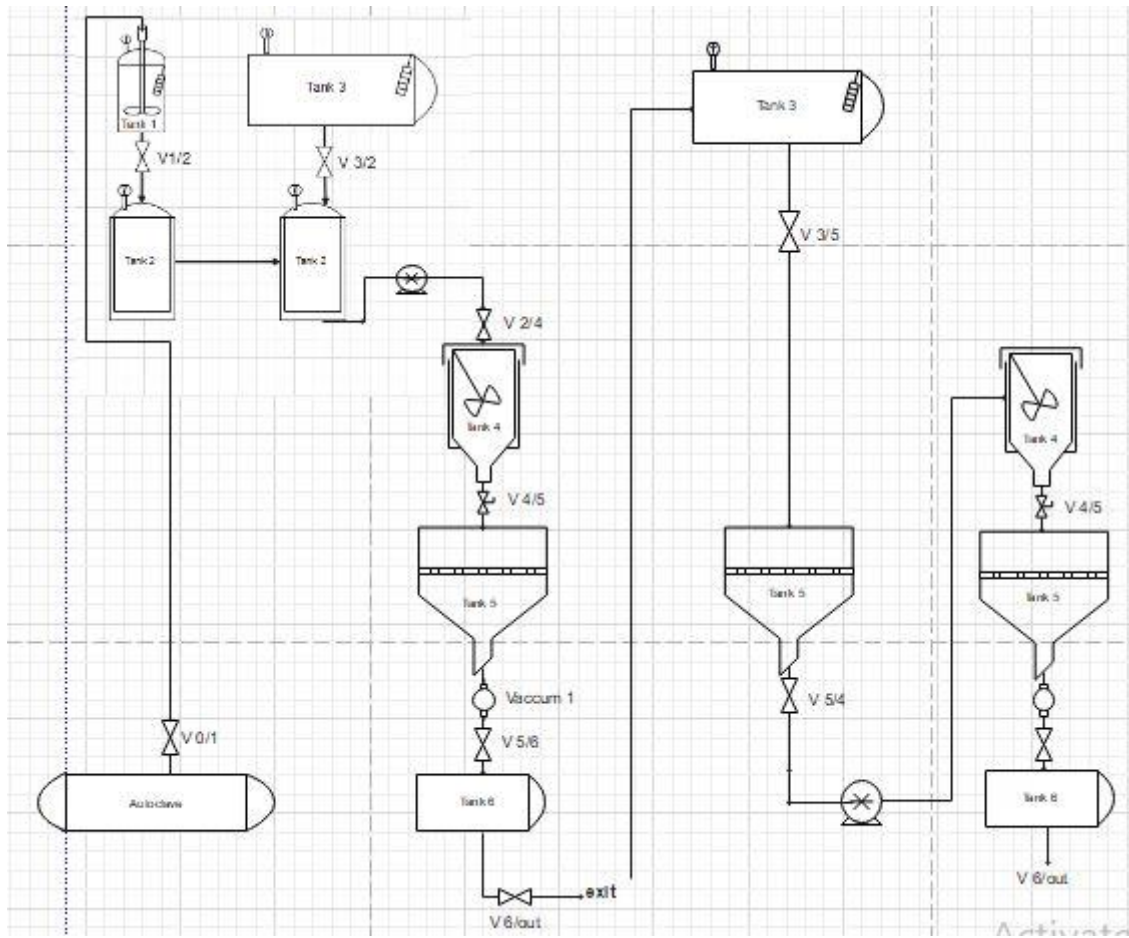


[02/18/2023 Word file contain the E-draw file of Arpproximate View for aspirin pilot plant](#)



word approxmative
view 18.02.23.docx

References:



[02/18/2023 word file contain the E-draw file of Dynamic view for aspirin pilot plant](#)



Word dynamic view
18.2.23.docx

5.3 Test specification

Autoclave System Test Specification:

Pre-Starting

Please read these instructions thoroughly. This will make sure you obtain full safe use, Keep this instruction manual in a handy place for future reference.

Filling the tank

1. Make sure all valves are closed
2. Make sure the power is turned off
3. Connect the water valve to autoclave
4. Open the water to fill the tank (amount of water should be between 60-70%)
5. Close the valve for water filling

Safety precaution

The hot water (121 °c) could suffer of second-degree burns

Autoclave operation method

1-Ensure all sanitary connections

2-Fill the autoclave tank with water

3-Plug the control system to electricity

4-check the control system if it works properly (Check if the valves works by turning them ON and OFF)

5-Operate the heater

6- Wait till the water transforms to steam ($T= 121^{\circ}\text{C}$ / $P= 20000$ Pa), operate the autoclave valve system to open

7-After finishing, Operate the pipe to open (to decrease the pressure)

System testcases

001: test the resistor of the autoclave

Step	Step Description	Expected Result
Precondition	System is off	

References:

Switch on the system	Turn on the heater	The water degree starts to going up to reach 121°C
Switch off the system.	Turn off the heater	The water will remain warm and could last for 5-6h
Postcondition	System is off	

002: AUTOCLAVE SYSTEME TEST OF PENICILLIN PRODUCTION

Step	Step Description	Expected Result
Precondition	System is OFF	
open/close the valves	open all valves from the control panel and reclosed them after few minutes	Release the air from the system
Switch on the heater	Turn on the heater from the control panel	THE SYSTEM IS heating the water till reach 121°C
Sterilization of the fermenter	Open the autoclave valve (autolave--->Autoclave valve)	The steam is filling the fermenter
Sterilization of the whole system	Open the fermenter valve to sterilize last 2 tanks (autolave--->fermenter--->fermenter valve)	Pressure should reach 2 at (20000 Pa) and temperature 121 °C
Open the manual discharge valve	Open the discharge valve manually	Pressure should decrease the steam vent off the system
Close the valves	close ALL the valves from the control panel and the discharge valve manually	ALL the valves are closed
switching OFF the system	switch off the system	the system is OFF
Postcondition	system is OFF	

Aspirin Production System Test Specification:

Pre-Starting

Please read these instructions thoroughly. This will make sure you obtain full safe use, Keep this instruction manual in a handy place for future reference.

Prepare the reactor

1. Make sure all valves are closed
2. Make sure the power is turned off
3. Connect the reactif valve to the reactor
4. Put the amount needed of the reactifs in the reactor (amount of reactifs: 1013.5g of salicylic acid, 2.5L acetic anhydride and 125ml concentrated H₂SO₄)
5. Closed the valve for reactifs filling.

Safety precaution

-The hot water (80 °c) could suffer some burns (tank number 2)

-Concentrated Sulfuric acid causes severe skin burns and eye damage. (Wear protective gloves/protective clothing/eye protection/face protection).

Aspirin Production operation method

1-Ensure all sanitary connections

2-Put the reactifs in the reactor (salicylic acid, acetic anhydride and H₂SO₄)

3-Plug the control system

4-check the control system if it's works properly

5-Operate the mixer to mix the reactifs in the reactor

6-Operate to add warm water to the mixture

7-Open the valves to reach the filtration system

References:

8-Operate to open the vacuum pump in the filtration system

9-Operate the valves to refiltration the aspirin

7-After finishing, Operate the pipe to close.

System testcases

003: AUTOCLAVE SYSTEME TEST OF ASPIRIN PRODUCTION

Step	Step Description	Expected Result
Precondition	System is off	
open/close the valves	open all valves from the control panel and reclosed them after few minutes	The air is Released from the system
Switch ON the heater	Turn on the heater from the control panel	THE SYSTEM IS heating the water till reach 134°C
Sterilization of the reactor (tank 1)	Open the autoclave valve (autolave--->Autoclave valve)	The steam is filling the reactor (tank 1) P= 2 Bar (2000 mBar)/T= 134 °C
Sterilization of the cooler (tank 2)	Open the reactor valve V1 (tank 1) to sterilize the cooler (autolave--->Reactor--->Reactor valve V1)	The steam is filling the reactor (tank 1) and the cooler (tank 2) P= 2 Bar (2000 mBar)/T= 134 °C
Sterilization the tank 3 (HOT WATER TANK)	Open the tank 3 (HOT WATER TANK) valve V6 to sterilize tank 3 (HOT WATER TANK---->HOT WATER TANK valve V6)	The steam is filling the reactor (tank 1), the cooler (tank 2)and the HOT WATER TANK (tank 3) P= 2 Bar (2000 mBar)/T= 134 °C

Sterilization of the crystallizer (tank 4)	Open the tank 2 (cooler) valve V2 to sterilize tank 4 (crystallizer) (crystallizer---->crystallizer valve V2)	The steam is filling the reactor (tank 1), the cooler (tank 2), the HOT WATER TANK (tank 3) and the crystallizer (tank 4) P= 2 Bar (2000 mBar)/T= 134 °C
Sterilization of the vacuum filter (tank 5)	Open the valve V3 to sterilize tank 4 (Crystallizer) to the tank 5 (Filter) (crystallizer---->crystallizer valve V3)	The steam is filling the reactor (tank 1), the cooler (tank 2), the HOT WATER TANK (tank 3), the crystallizer (tank 4) and the vacuum filter (tank 5) P= 2 Bar (2000 mBar)/T= 134 °C
Sterilization of the waste tank (tank 6)	Open the valve V4 tank 5 (Filter) to sterilize tank 6 (Waste) (Filter---->Filter valve V4)	The steam is filling the reactor (tank 1), the cooler (tank 2), the HOT WATER TANK (tank 3), the crystallizer (tank 4), the vacuum filter (tank 5) and the waste tank (tank 6) P= 2 Bar (2000 mBar)/T= 134 °C
Open the manual discharge valve	Open the discharge valve manually	Pressure should decrease and the steam vent off the system
Switch OFF the heater	Turn OFF the heater from the control panel	the heater is OFF
CLose ALL the valves	close ALL the valves from the control panel and the discharge valve manually	ALL the valves are closed
switching OFF the system	switch off the system	the system is OFF
Postcondition	system is OFF	

004: ASPIRIN PRIDOUCTION SYSTEME TEST

Step	Step Description	Expected Result
Precondition	System is OFF	
TURNING ON the system	Turn ON the control panel	The system is ON
Switch on the mixer 1 (tank 1: Reactor)	Turn on the mixer 1 from the control panel (Reactor--->Mixer ON)	Mixing the reagents to obtain the mixture in the reactor (tank 1)
Open the valve V1 (tank1 : Reactor)	Open the valve V1 to transfer the mixture from tank 1 (reactor) to tank 2 (cooler) (Reactor---->Reactor valve V1)	The mixture is transferred to cooler (tank 2)
Open the valve 6 (tank 3 : HOT WATER)	Open the valve V6 to add water from HOT WATER TANK (tank 3) to the cooler (tank 2) (HOt WATER TANK---->HOT WATER TANK valve V6)	The warm water is in the cooler (tank 2) with the mixture
Open the valve V2 (tank 2: Cooler)	Turn ON the pump 1 Open the valve V2 to transfer the Water from cooler (tank 2) to crystallizer (tank 4) (cooler---->cooler pump 1) (cooler---->cooler valve V2)	The reagents are transferred to the crystallizer (tank 4) and formation of crystals
Switch on the mixer 2 (tank 4 : Crystallizer)	Turn on the mixer from the control panel (Crystallizer--->Mixer ON)	Mixer 2 is ON and start Mixing the reagents in the Crystallizer (tank 4)
Open the valve V3 (tank 4: Crystallizer)	Open the valve V3 to transfer the Water from crystallizer (tank 4) to the filter (tank 5)	The mixture transferred to the filter (tank 5)

	(crystallizer---->crystallizer valve V3)	
Switch on the vacuum pump	Turn on the vacuum pump from the control panel (Filter---->Filter pump ON)	The air is vacuumed in tank 6 (Waste tank)
Open the valve V4 (tank 5 : Filter)	Open the valve V4: 1-the vacuum filtration begin 2-Aspirin crystals are now in the filter (tank 5) (Filter---->Filter valve V5)	The filtrate transferred to the waste tank (tank 6)
Open the valve V7	Open the valve V7 to add 30L warm water from WARM WATER TANK (tank 3) to vacuum filter (tank 5) (WARM WATER TANK---->WARM WATER Valve V7)	Crystals re-dissolved with 30L warm water after 20min in the vacuum filter (tank 5)
Open the valve V5 and pump 2 (tank 5 : Filter)	Open the valve V5 The solution is pumped from tank 5 to tank 4 for the second crystallization (vacuum filter---->vacuum filter Valve V5)	Solution transferred to the crystallizer (tank 4) and start the crystallization
Switch on the vacuum pump for the second filtration	Turn on the vacuum pump from the control panel (Filter---->vacuum pump ON)	The air is partial vacuumed in tank 6
Open the valve V3 and V4	Open the valve V3 and V4: 1-the vacuum filtration begin 2-Aspirin crystals are now in the vacuum filter (tank 5) (1-crystallizer---->crystallizer valve V3) (2-vacuum filter---->vacuum filter valve V4)	The filtrate transferred to the waste tank (tank 6)
switching OFF the system	switch OFF the system	the system is OFF

References:

Postcondition

system is OFF

5.4 Aspirine:

5.4.1 Requirements For Aspirin Pilot Plant Production

System requirements :

- Aspirin Pilot Plant shall be able to produce the aspirin.
- The control panel shall be able to control all the pumps, valves, mixers and read the data of the sensors (Temperature-pressure).

Physical requirements :

- The pipes shall be able to withstand the temperatures and pressures that exist at the points.
- Temperature that shall be withstood: 80 degrees celsius.
- Pressure that shall be withstood: 2 bar.
- The tanks shall be able to withstand the Temperatures exchanges, pressures and mechanical forces that exist at the points.
- Temperature that shall be withstood: 80+ degrees celsius.
- Pressure that shall be withstood: 2 bar.
- mechanical force: mixer movements and rotation.

Chemical requirements :

- The Tanks system shall be able to insulate the chemical reagents.
- The Tanks system shall be able to withstand the corrosion with H₂SO₄ and acetic acid.
- The pipe system used shall be able to withstand the corrosion with H₂SO₄ and acetic acid.
- The valves shall be able to withstand the corrosion with H₂SO₄ and acetic acid.

Mechanical requirements :

References:

- The Tank system shall be made of Stainless Steel 316.
- The Tank system shall be able to close the system completely.
- The pipes shall be made of stainless steel 316.
- The pipes shall be able to resist the pressure without let gas exit through.
- The valves shall be made of stainless steel 316.
- The valves shall be able to close completely.
- The valves shall be able to open or close with independent pressure.
- The aspirin pilot plant shall be designed according to the mechanical design.

Safety requirements :

- The system shall be enclosed completely to ensure no toxic gas and to avoid contact with the burns from concentrated acid (H₂SO₄).
- The system shall be enclosed during the sterilization step to avoid the burns form hot vapor used to sterilization.

5.4.2 Aspirin pilot plant Mechanical Realisation

Stand installation : (18/01/2023-
07/04/2023)

References:





References:

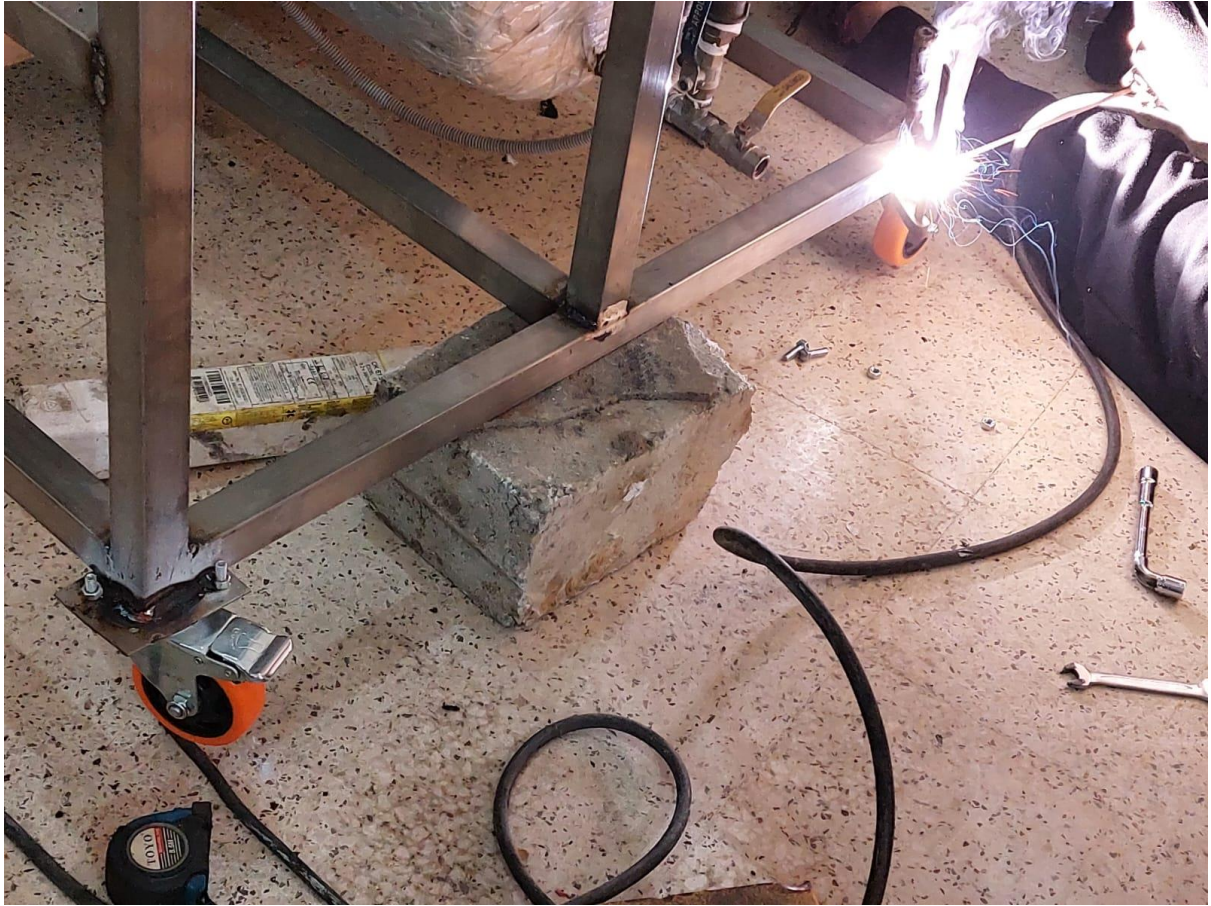




Wheel stand installation :(19/01/2023)

References:





References:



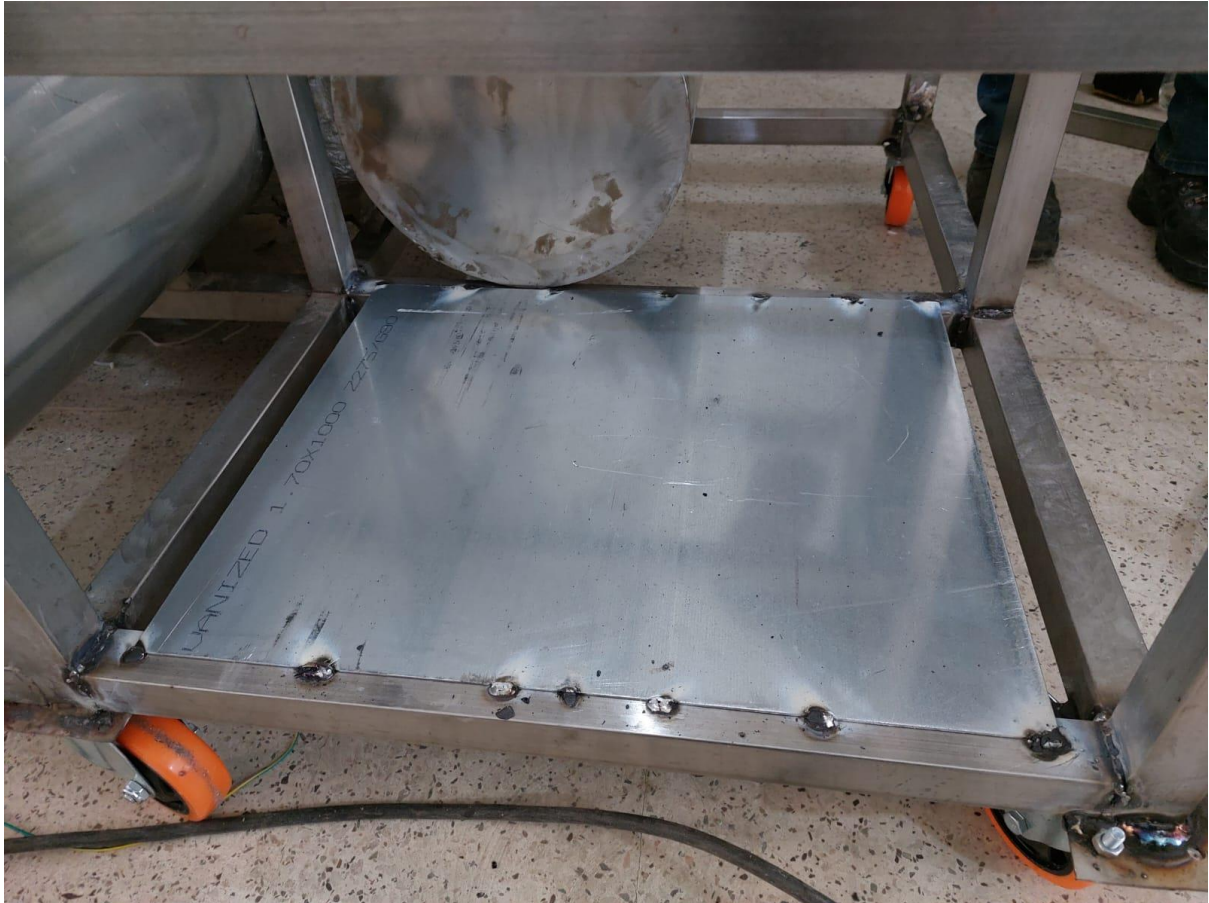


References:





References:



Autoclave place editing :(20/01/2023)



References:





References:

Bath for tank 2 (cooler) + tank 3
(crystallizer) and testing :(22/01/2023)-
(04/07/2023)



References:





References:





References:





References:

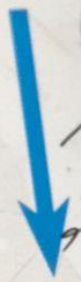


Crystallizer and Filter
equipements:(07/03/2023)



References:





A1-1
2120
1979

A. B. S. METALS
A. B. S. METALS COMPANY



STAINLESS STEEL SERVICE GROUP



A. B. S. METALS
A. B. S. METALS COMPANY

A. B. S. METALS
A. B. S. METALS COMPANY

A. B. S. METALS
A. B. S. METALS COMPANY

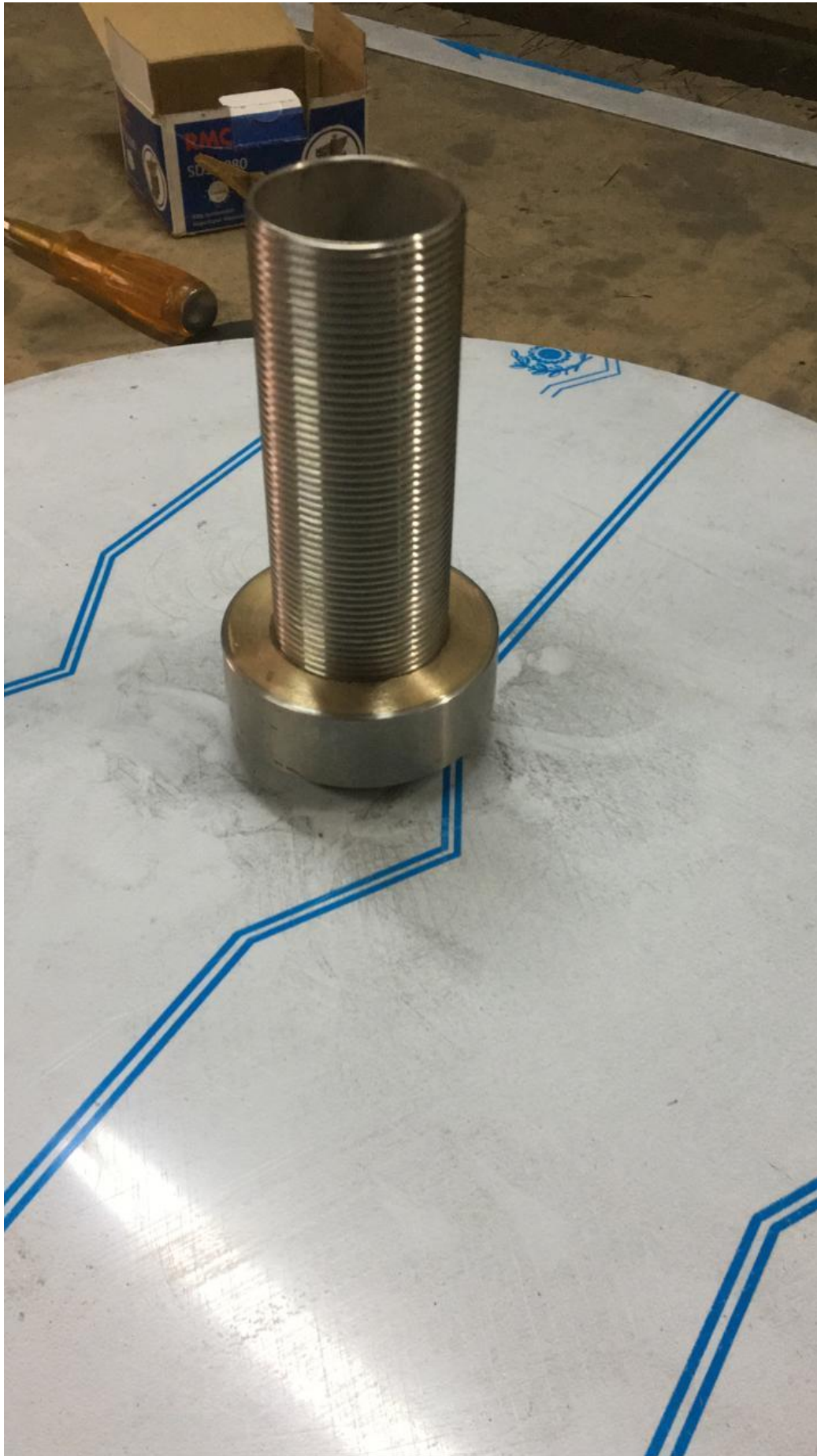
References:



Cover for vacuum filter :(09/03/2023)

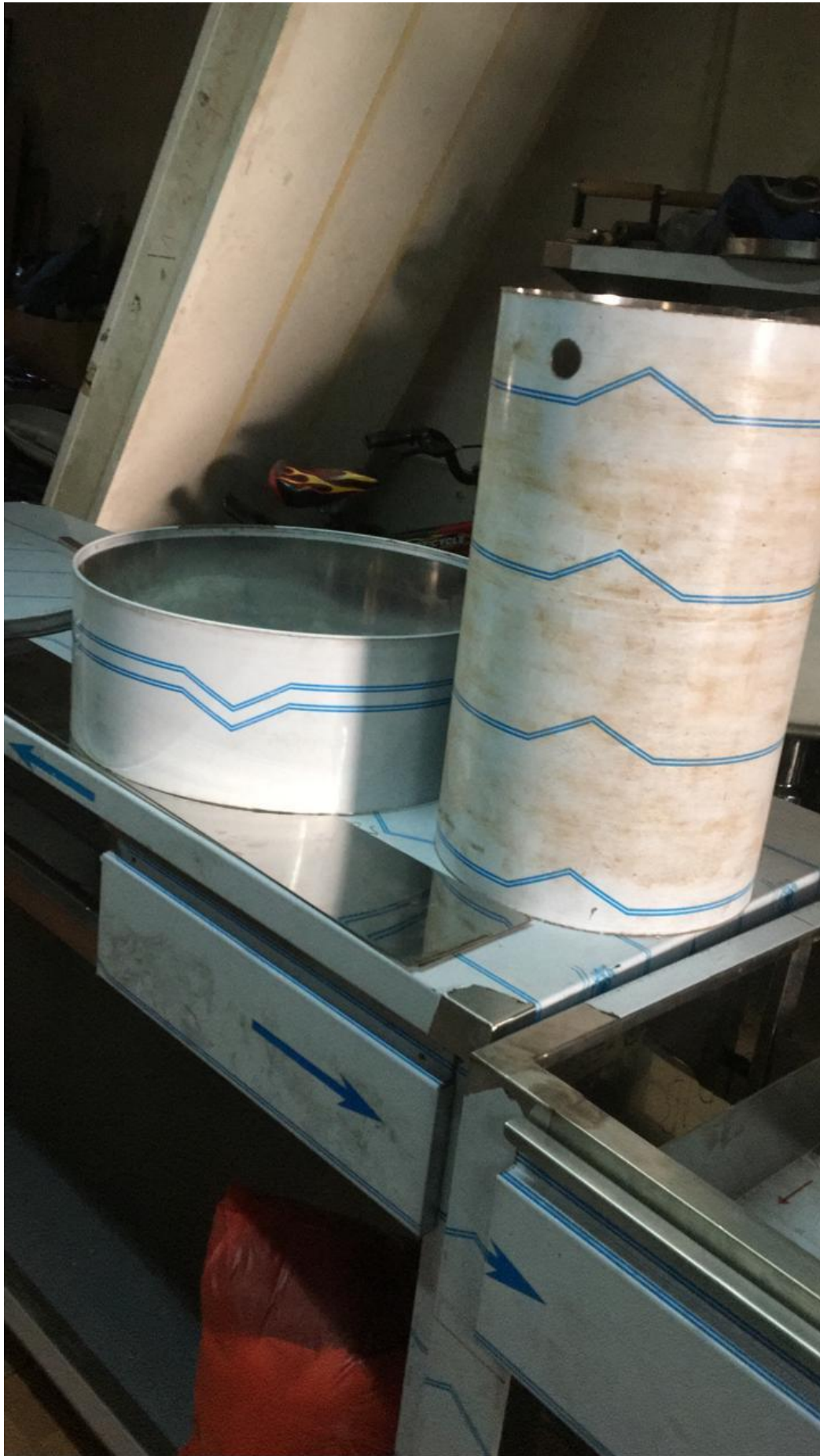
References:





References:

crystallizer tank and the filter tank
:(09/03/2023)



Sieve and the filter con :(13/03/2023)





Vacuum filter realisation :(17/03/2023)

References:





References:



Crystallizer realisation :(21/03/2023-
07/04/2023)

References:





References:





References:



Tank 1 (The Heater)
realisation:(07/04/2023)

References:





Tank 6 (Waste Tank)
realisation:(07/04/2023)

References:



Pipes stainless steel
realisation:(02/05/2023)



References:





References:

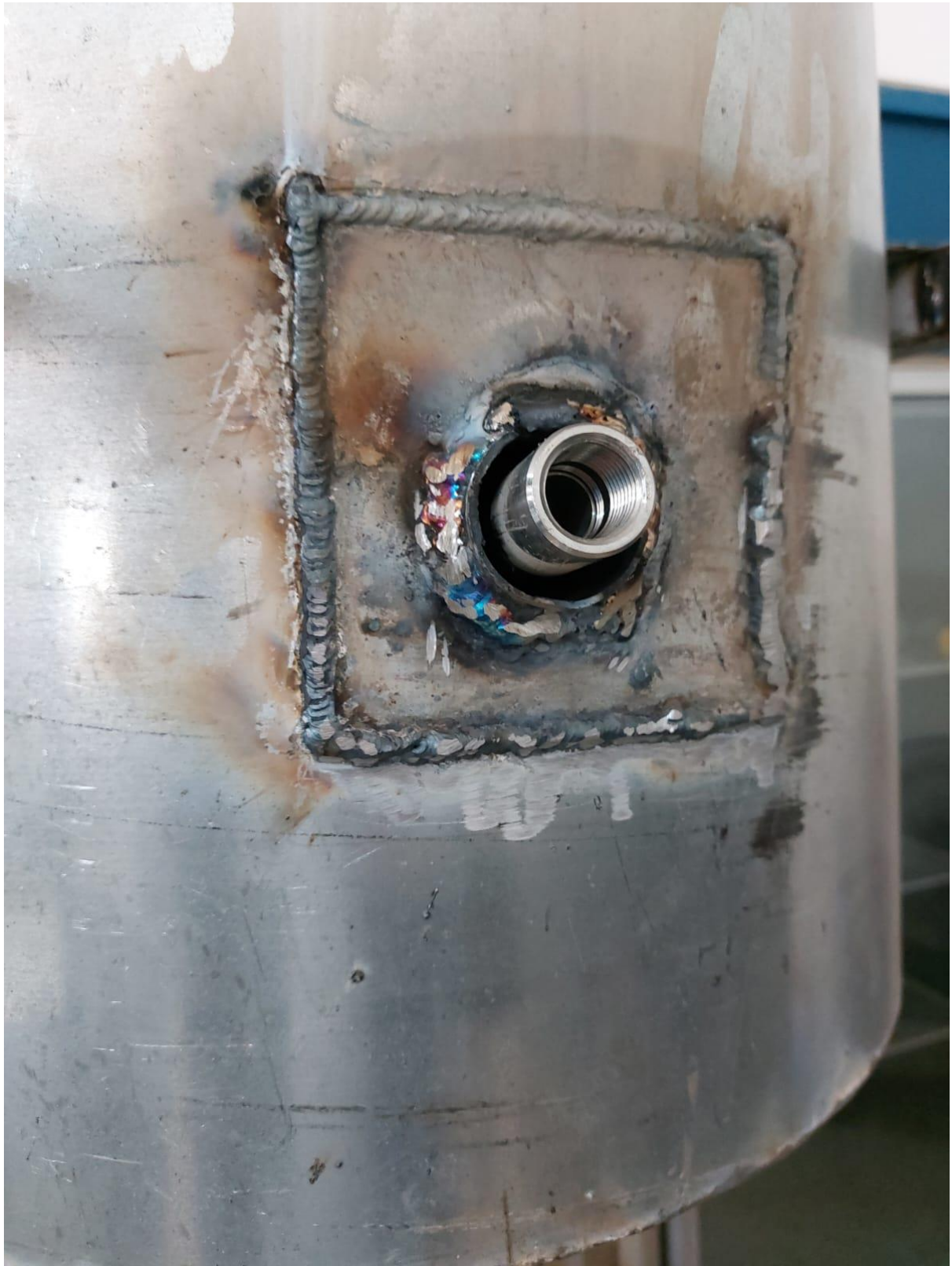


Sensors stainless steel
instalation:(02/05/2023)



References:







Reactor for aspirin:(20/10/2023)



References:





References:





References:



Pilot plant mechanical realization last
form: (11-13-2023)

References:



5.4.3 System tests

5.4.3.1 003: Aspirin Production System Test (Water test)

Step	Step Description	Results
Precondition	System is OFF	
TURNING ON the system	Turn ON the control panel	The system is ON
Switch on the mixer 1 (tank 1 : Reactor)	Turn on the mixer 1 from the control panel (Reactor--->Mixer ON)	Mixer 1 is ON and start Mixing the Water (Reactor)
Open the valve V1 (tank1 : Reactor)	Open the valve V1 to transfer the mixture from tank 1 (reactor) to tank 2 (cooler) (Reactor---->Reactor valve V1)	The Water is transferred to Cooler (tank 2)
Open the valve 6 (tank 3 : HOT WATER)	Open the valve V6 to add water from HOT WATER TANK (tank 3) to the cooler (tank 2) (HOT WATER TANK---->HOT WATER TANK valve V6)	The water is in the cooler (tank 2) PS: -leak in pipes from tank 3 (Hot water) to tank 2 (Cooler) -the flow sensor not working
Open the valve V2 (tank 2: Cooler)	Turn ON the pump 1 Open the valve V2 to transfer the Water from cooler (tank 2) to crystallizer (tank 4) (cooler---->cooler pump 1) (cooler---->cooler valve V2)	The Water are transferred to the crystallizer (tank 4) PS: pump 1 stopped pumping

References:

Switch on the mixer 2 (tank 4 : Crystallizer)	Turn on the mixer from the control panel (Crystallizer--->Mixer ON)	Mixer 2 is ON and start Mixing the Water Crystallizer (tank 4)
Open the valve V3 (tank 4: Crystallizer)	Open the valve V3 to transfer the Water from crystallizer (tank 4) to the filter (tank 5) (crystallizer---->crystallizer valve V3)	The Water transferred to the filter (tank 5)
Open the valve V7 (TANK 3: HOT WATER)	Open the valve V7 to add water from HOT WATER TANK (tank 3) to filter tank(tank 5) (HOT WATER TANK---->HOT WATER Valve V7)	The Water transferred to filter tank (tank 5)
Open the valve V5 (tank 5: Filter)	Turn ON the pump 2 Open the valve V5 The Water is pumped from tank 5 to tank 4 for the second crystallization (filter---->filter Valve V5)	Water transferred to the crystallizer (tank 4) PS: pump 2 stopped pumping
switching OFF the system	switch OFF the system	the system is OFF
Postcondition	system is OFF	

Overall Review: In this test we've noticed some leaks in some pipes, pumps stopping from pumping and 1 Temperature sensor need to reprogrammed

in the next test we will fix all those problems and try another test with water only.

[Video003 \(1\): Testing Valves and mixers \(water test 1\) 28/11/2023](#)

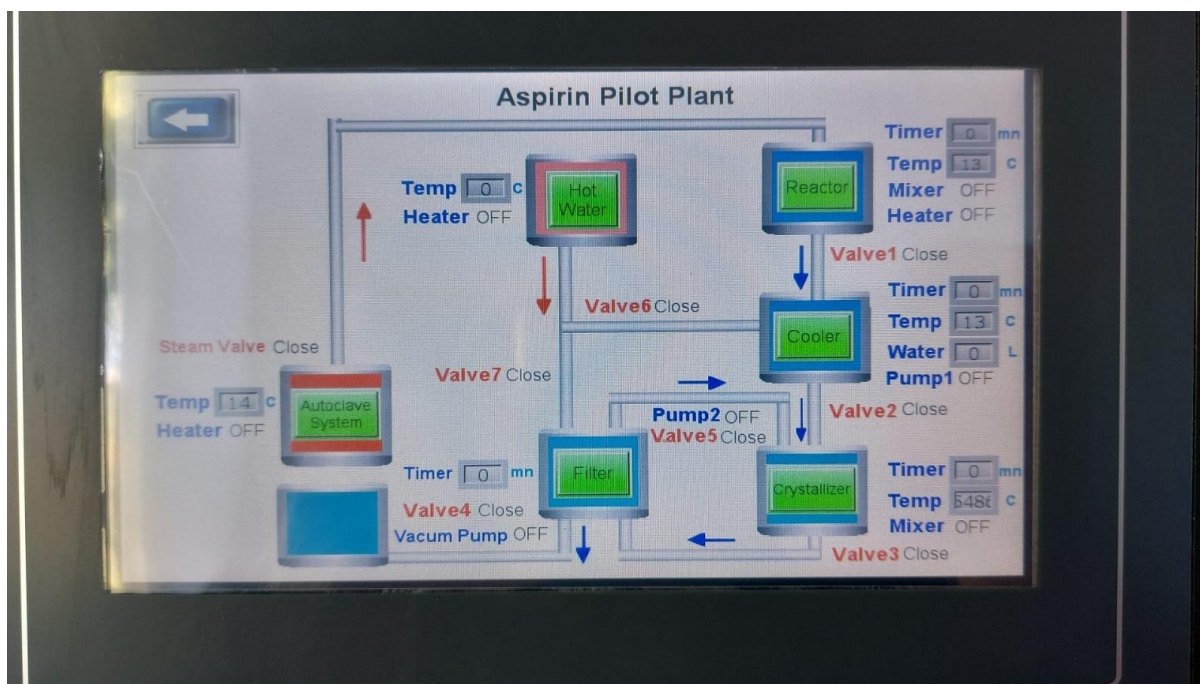


WhatsApp Video
2023-11-30 at 9.36.27

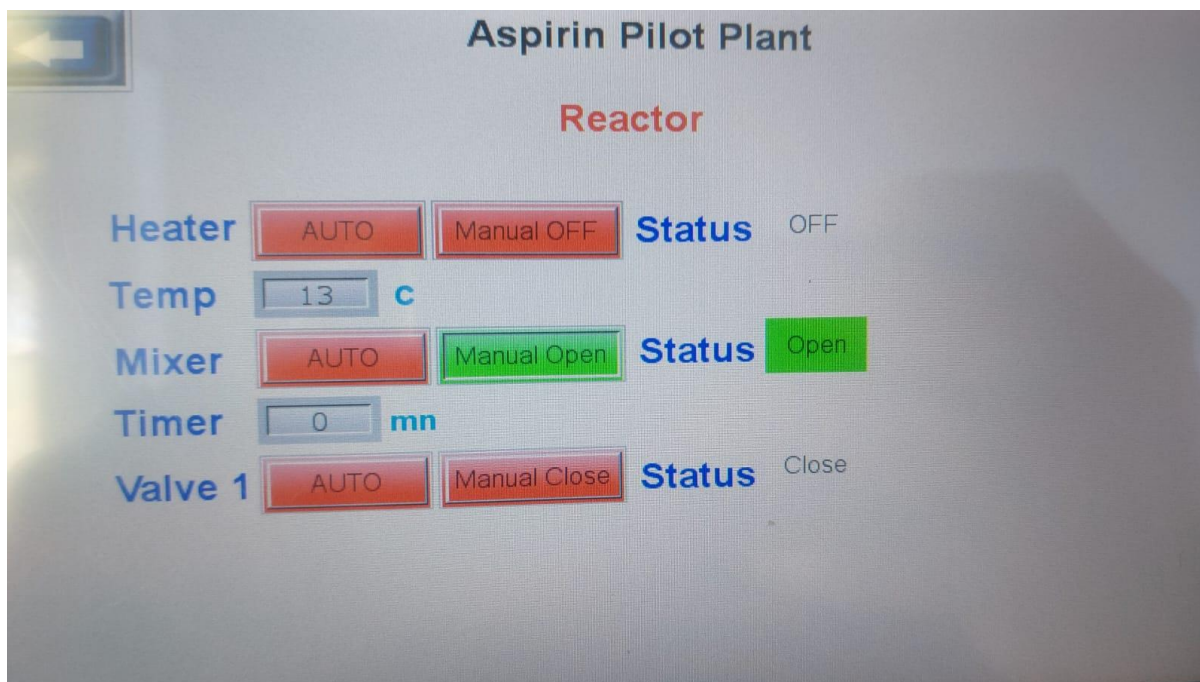
[Video003 \(2\): Testing Valves and mixers and sensors \(water test 2\) 28/11/2023](#)



Video 2 water test
28.11.23.mp4

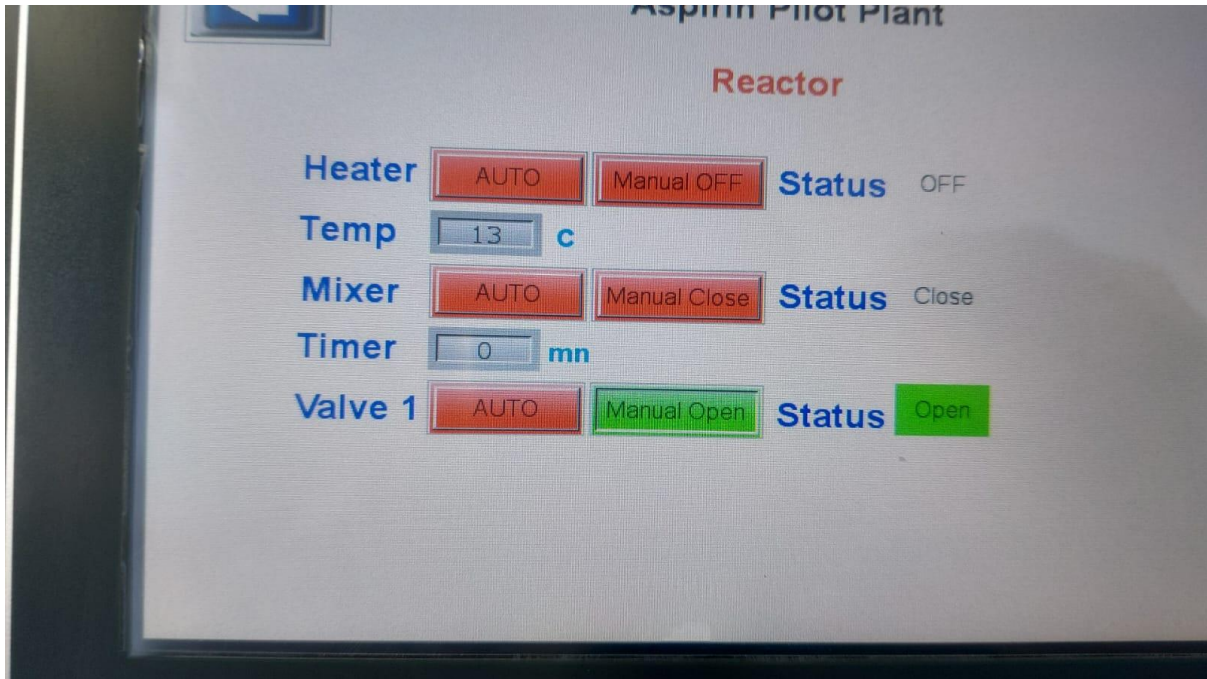


1. Overview of control panel system for Aspirin pilot plant

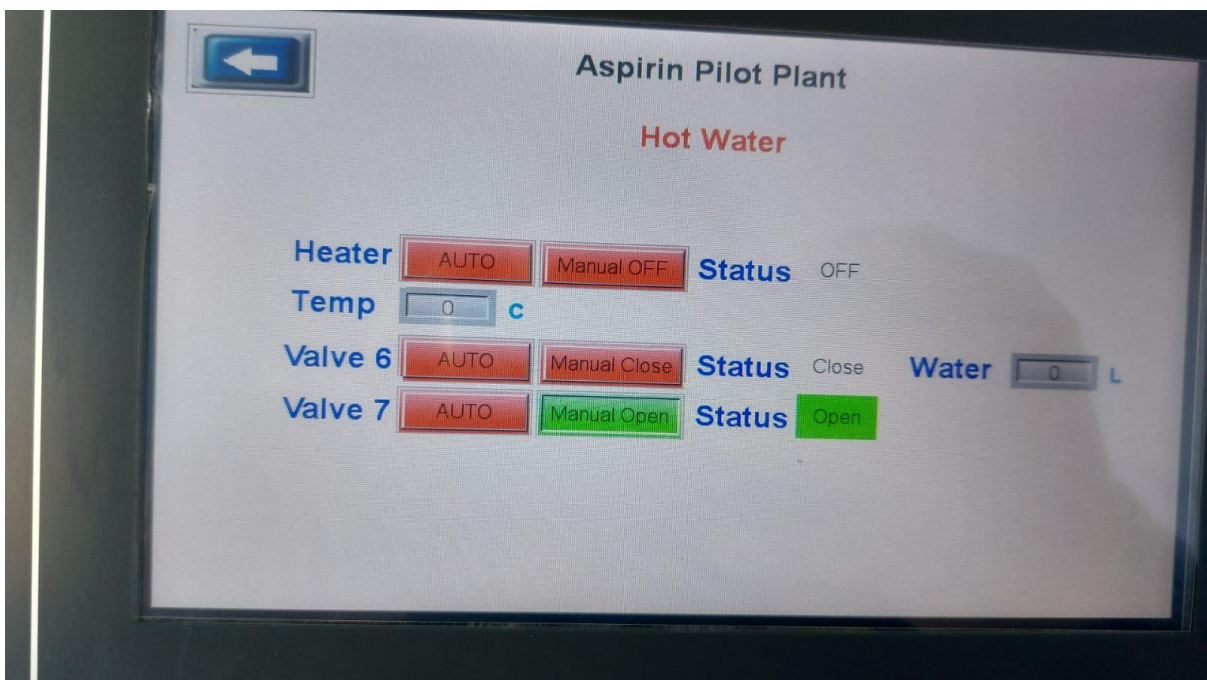


References:

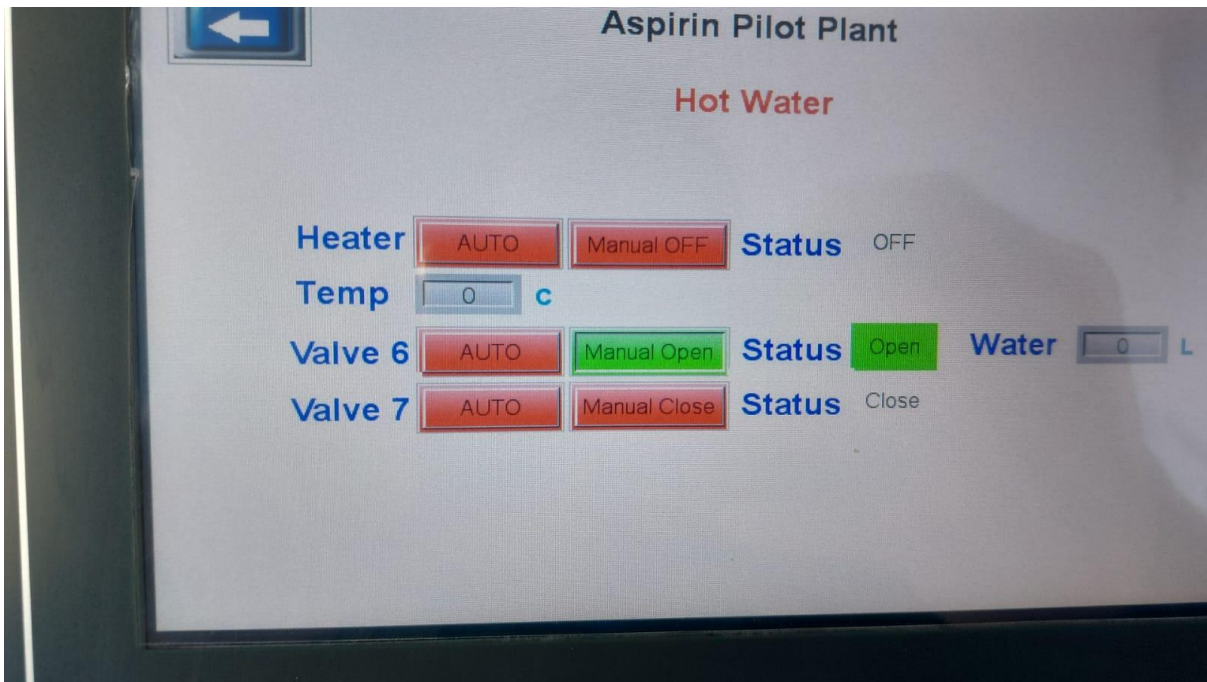
2. Turn ON the mixer by switching manually to mix the solution (PS: in this test we used water only)



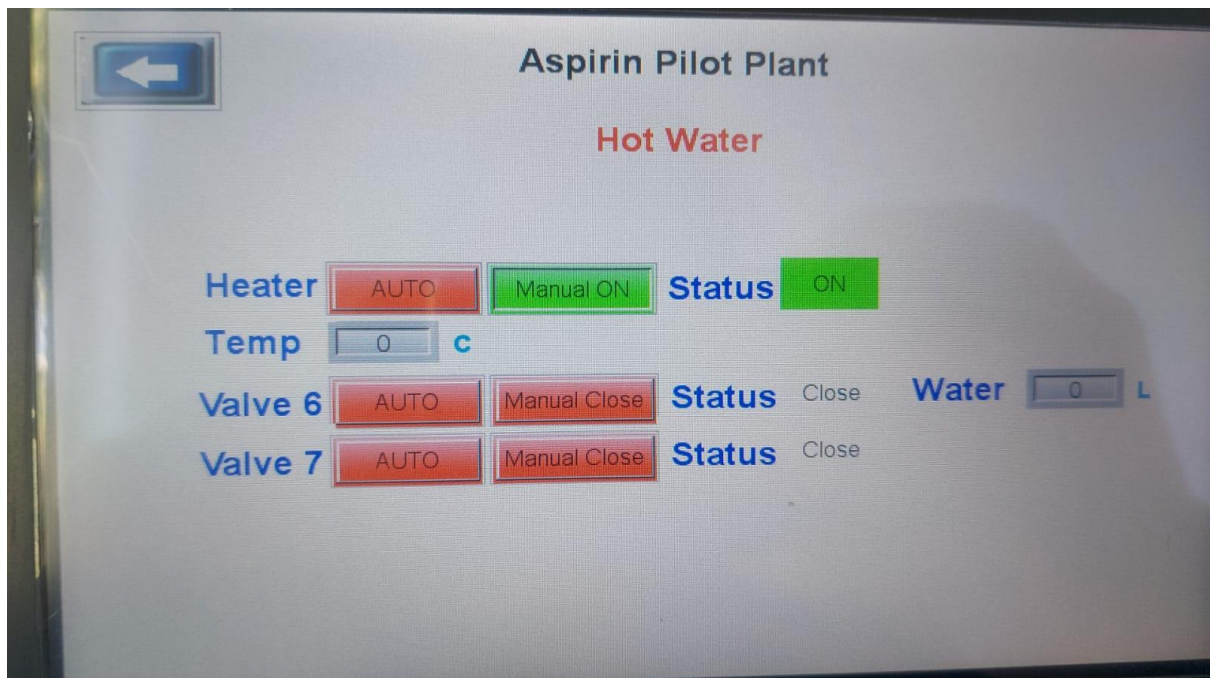
3. Turn ON manually valve 1 to transfer the solution to the Cooler (tank 3)



4. Turn ON manually valve 7 to transfer distilled water from Hot water tank (tank 2) to filter (tank 5)

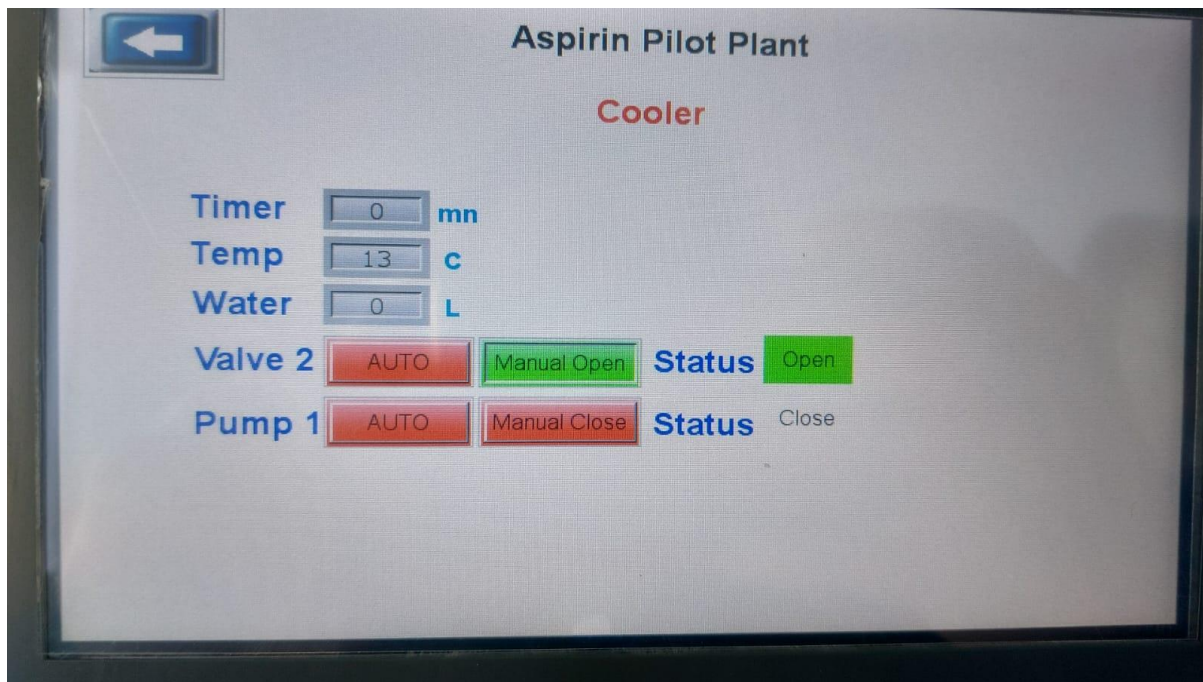


5. Turn ON manually valve 6 to transfer distilled water from Hot water tank (tank 2) to Cooler (tank 3) (PS: there's a flow sensor to determine the quantity needed of distilled water)

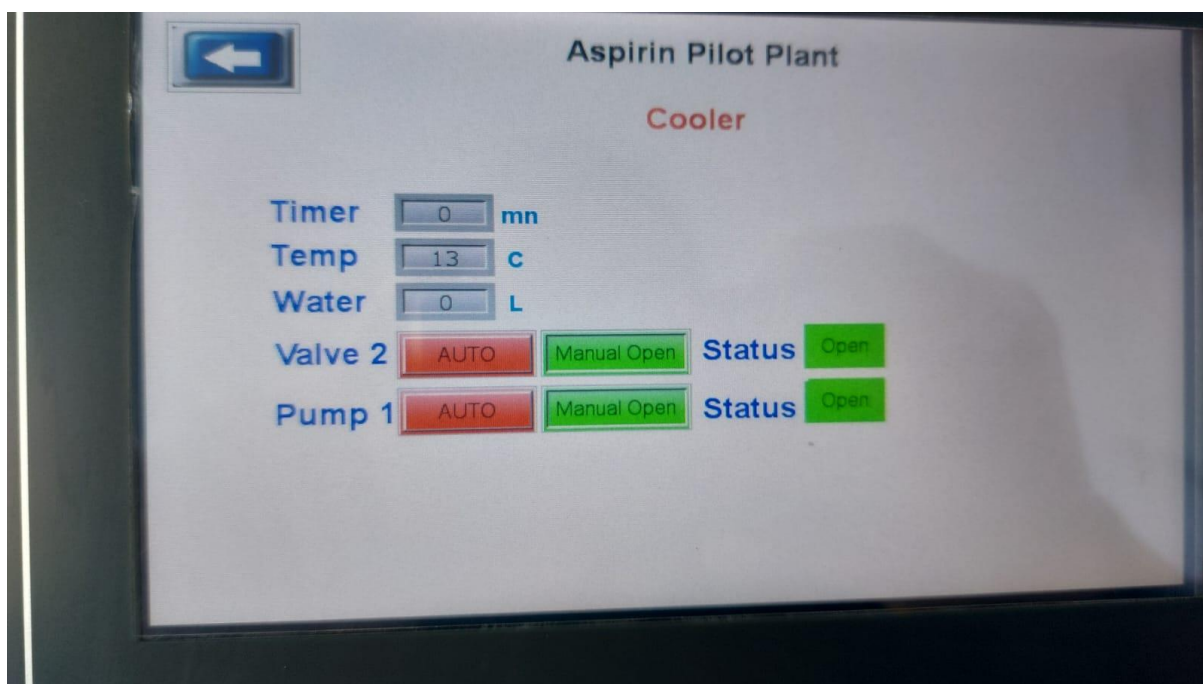


6. Turn ON manually the heater to warm up distilled water needed (PS: there's a temperature sensor to determine the temperature of DW required)

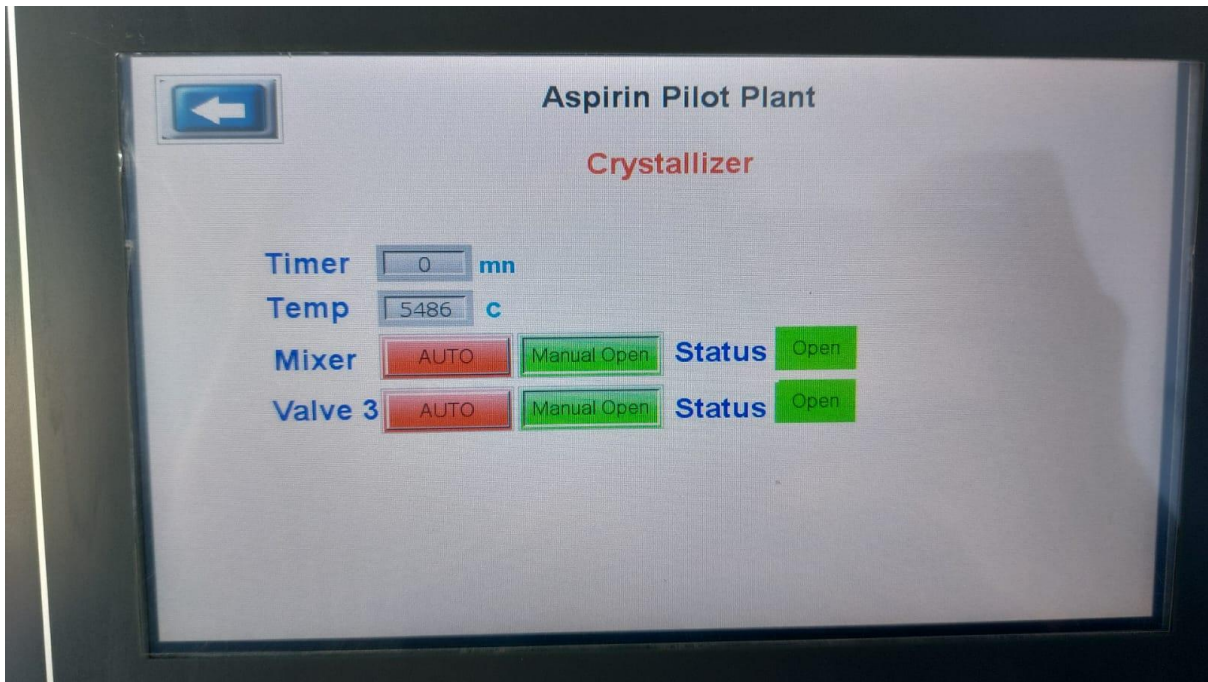
References:



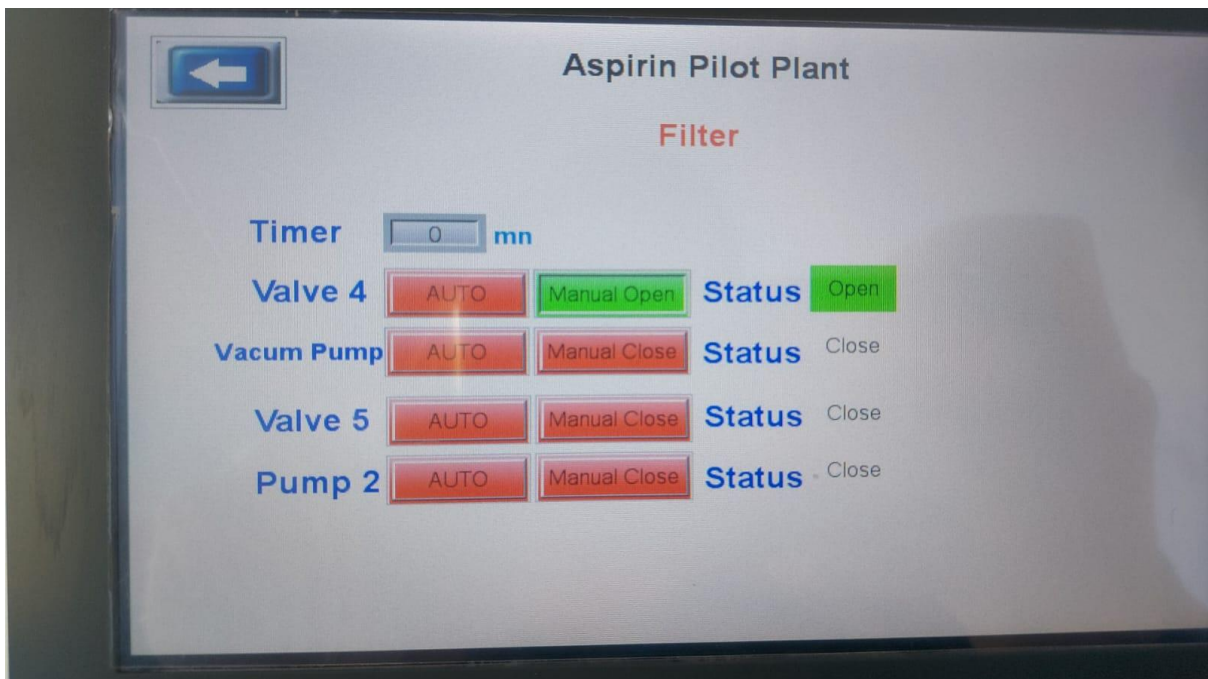
7. Turn ON manually valve 2 to transfer the mixture (Water) from the cooler (tank 3) to crystallizer (tank 4)



8. Turn ON manually pump 1 to transfer the mixture (Water) from the cooler (tank 3) to crystallizer (tank 4)

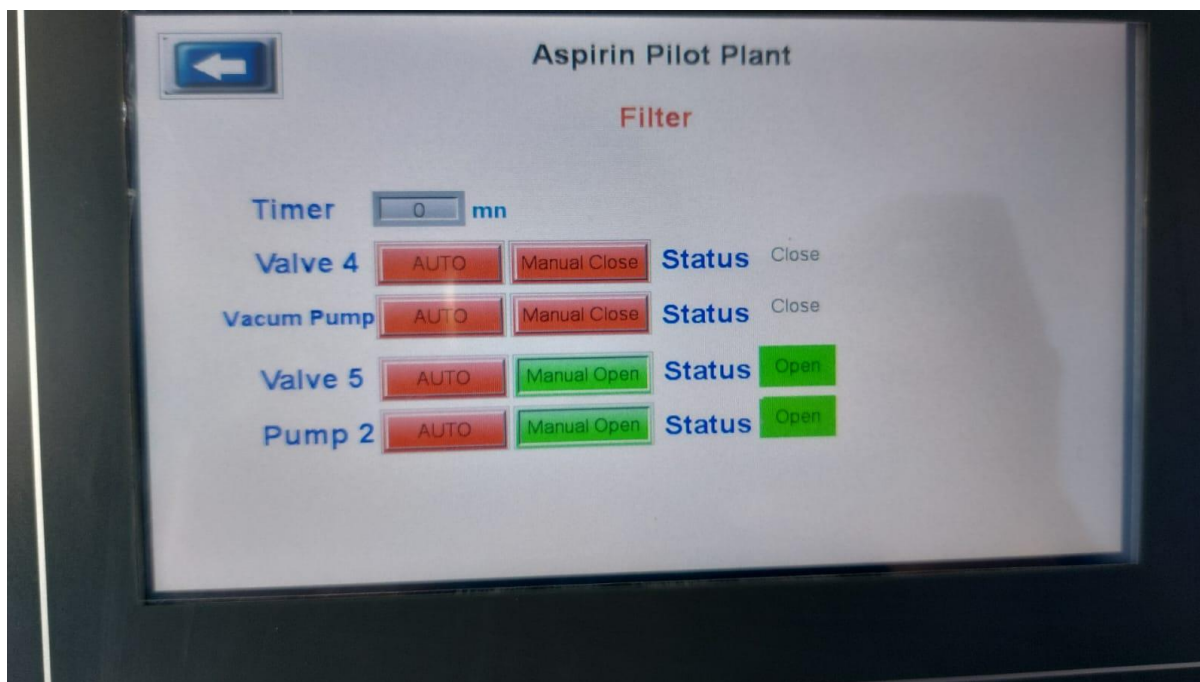


9. Turn ON manually the "Mixer" and valve 3 to mixing and transfer the mixture from the Crystallizer (tank 4) to the Filter (tank 5)



10. Turn ON the valve 4 to transfer the mixture (water) from the Filter (tank 5) to Waste tank (tank 6)

References:



11. Turn ON valve 5 and pump 2 to transfer the mixture (water) from Filter (tank 5) to crystallizer (tank 4) to recrystallization (PURIFICATION STEP)

5.4.3.2 004: Aspirin Production System Test (Water test)

Step	Step Description	Results
Precondition	System is OFF	
TURNING ON the system	Turn ON the control panel	The system is ON
Switch on the mixer 1 (tank 1 : Reactor)	Turn on the mixer 1 from the control panel (Reactor--->Mixer ON)	Mixer 1 is ON and start Mixing the Water (Reactor)
Open the valve V1 (tank1 : Reactor)	Open the valve V1 to transfer the mixture from tank 1 (reactor) to tank 2 (cooler) (Reactor---->Reactor valve V1)	The Water is transferred to Cooler (tank 2)
Open the valve 6 (tank 3 : HOT WATER)	Open the valve V6 to add water from HOT WATER TANK (tank 3) to the cooler (tank 2) (HOT WATER TANK---->HOT WATER TANK valve V6)	-The water is in the cooler (tank 2) -the flow sensor not working
Open the valve V2 (tank 2: Cooler)	Turn ON the pump 1 Open the valve V2 to transfer the Water from cooler (tank 2) to crystallizer (tank 4) (cooler---->cooler pump 1) (cooler---->cooler valve V2)	The Water are transferred to the crystallizer (tank 4) PS: pump 1 stopped pumping
Switch on the mixer 2 (tank 4 : Crystallizer)	Turn on the mixer from the control panel (Crystallizer--->Mixer ON)	Mixer 2 is ON and start Mixing the Water Crystallizer (tank 4)

References:

Open the valve V3 (tank 4: Crystallizer)	Open the valve V3 to transfer the Water from crystallizer (tank 4) to the filter (tank 5) (crystallizer---->crystallizer valve V3)	The Water transferred to the filter (tank 5)
Open the valve V7 (TANK 3: HOT WATER)	Open the valve V7 to add water from HOT WATER TANK (tank 3) to filter tank(tank 5) (HOT WATER TANK---->HOT WATER Valve V7)	The Water transferred to filter tank (tank 5)
Open the valve V5 (tank 5: Filter)	Turn ON the pump 2 Open the valve V5 The Water is pumped from tank 5 to tank 4 for the second crystallization (filter---->filter Valve V5)	Water transferred to the crystallizer (tank 4) PS: pump 2 stopped pumping
switching OFF the system	switch OFF the system	the system is OFF
Postcondition	system is OFF	

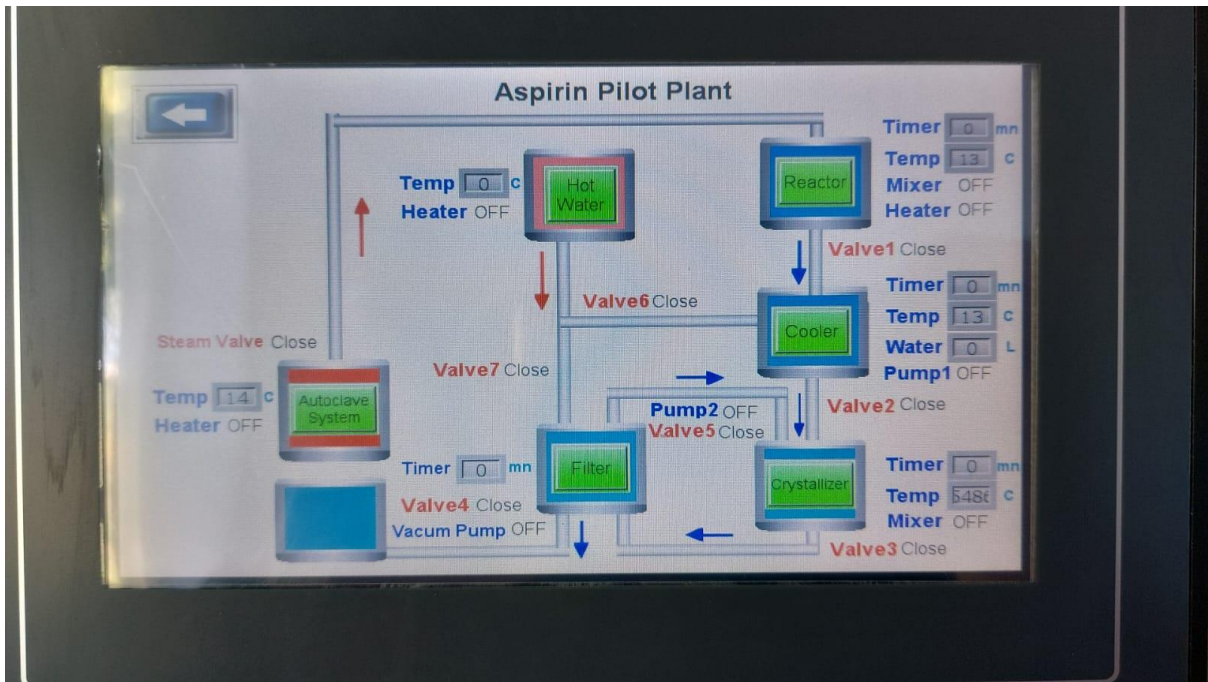
Overall Review: In this test we've noticed leak in HOT WATER TANK, pumps stopping from pumping (fixed confirmed), Temperature sensor brokeed need to release the pressure from the sensor and we need to make a vent for air pressure in tank 3 (Cooler).

in the next test we will fix all those problems and try another test with water only.

[Video004: Testing Valves, mixers and pumps \(water test 3\) 1/12/2023](#)



aspirin pilot plant
test water only 003 vic



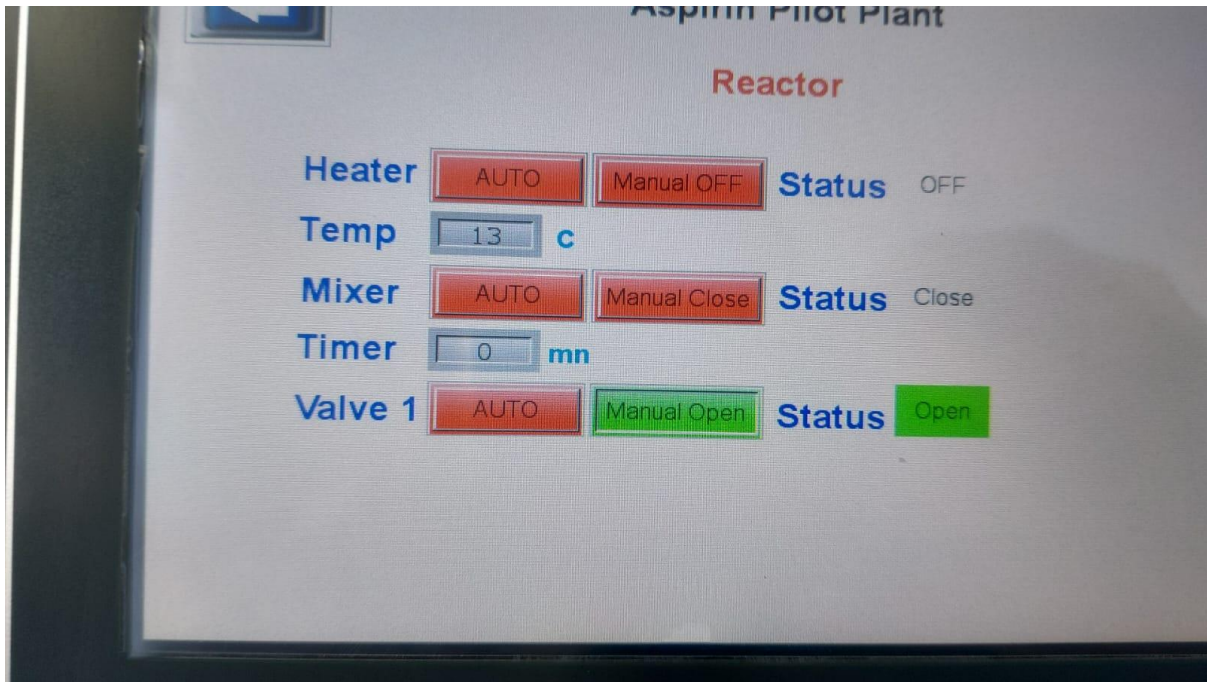
1. Overview of control panel system for Aspirin pilot plant

The screenshot shows the detailed control interface for the Reactor unit, titled "Aspirin Pilot Plant" and "Reactor". The controls are as follows:

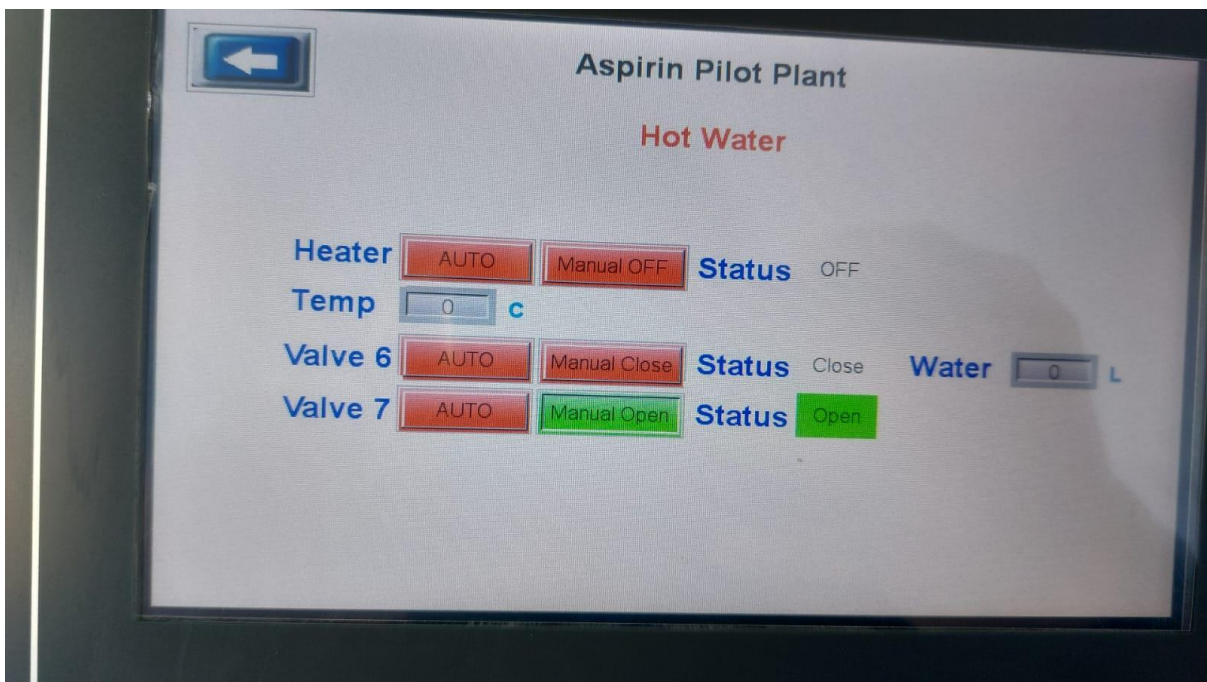
Heater	AUTO	Manual OFF	Status	OFF
Temp	13	c		
Mixer	AUTO	Manual Open	Status	Open
Timer	0	mn		
Valve 1	AUTO	Manual Close	Status	Close

2. Turn ON the mixer by switching manually to mix the solution (PS: in this test we used water only)

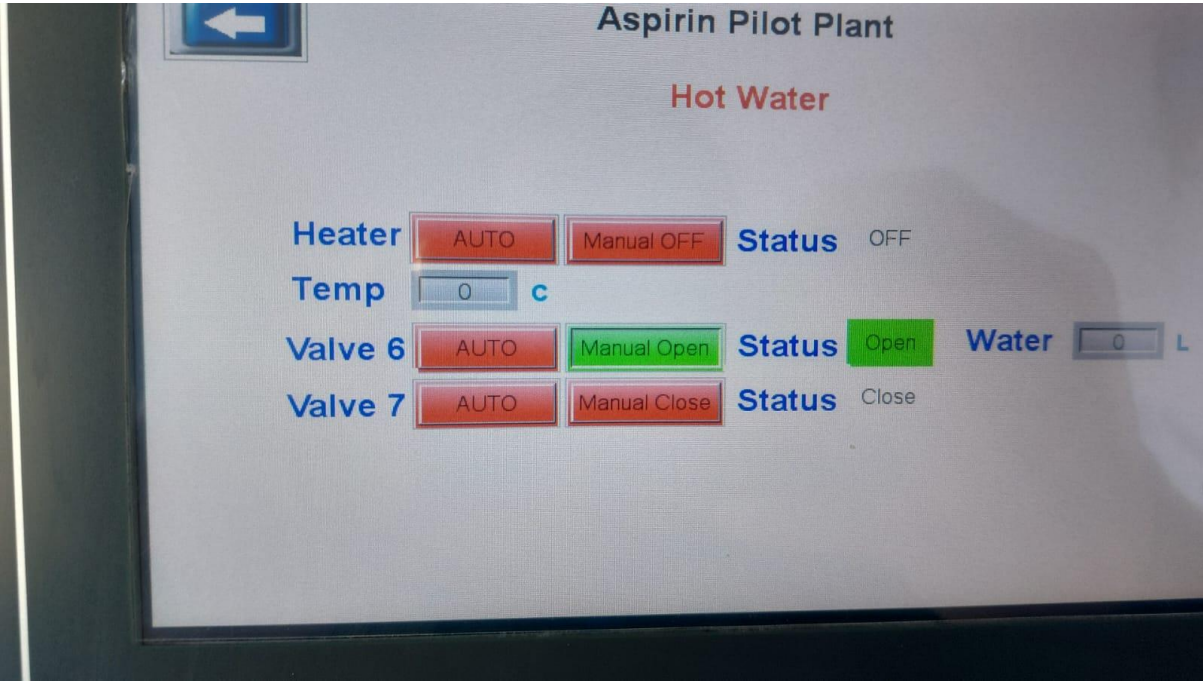
References:



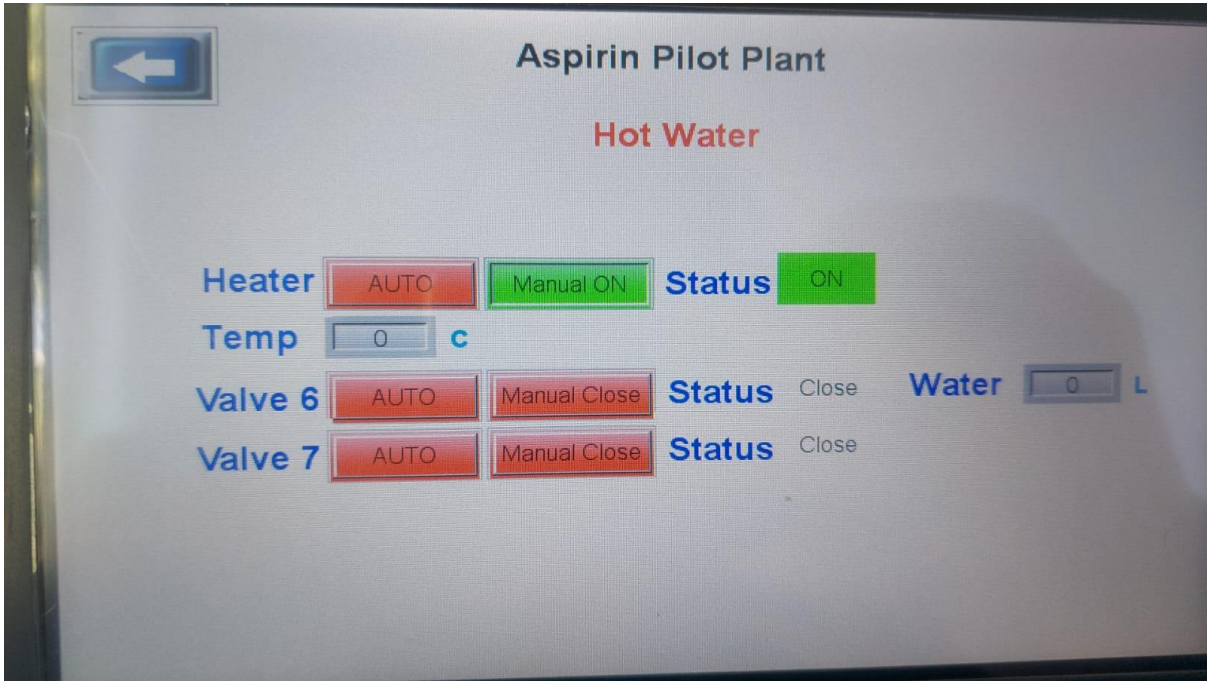
3. Turn ON manually valve 1 to transfer the solution to the Cooler (tank 3)



4. Turn ON manually valve 7 to transfer distilled water from Hot water tank (tank 2) to filter (tank 5)

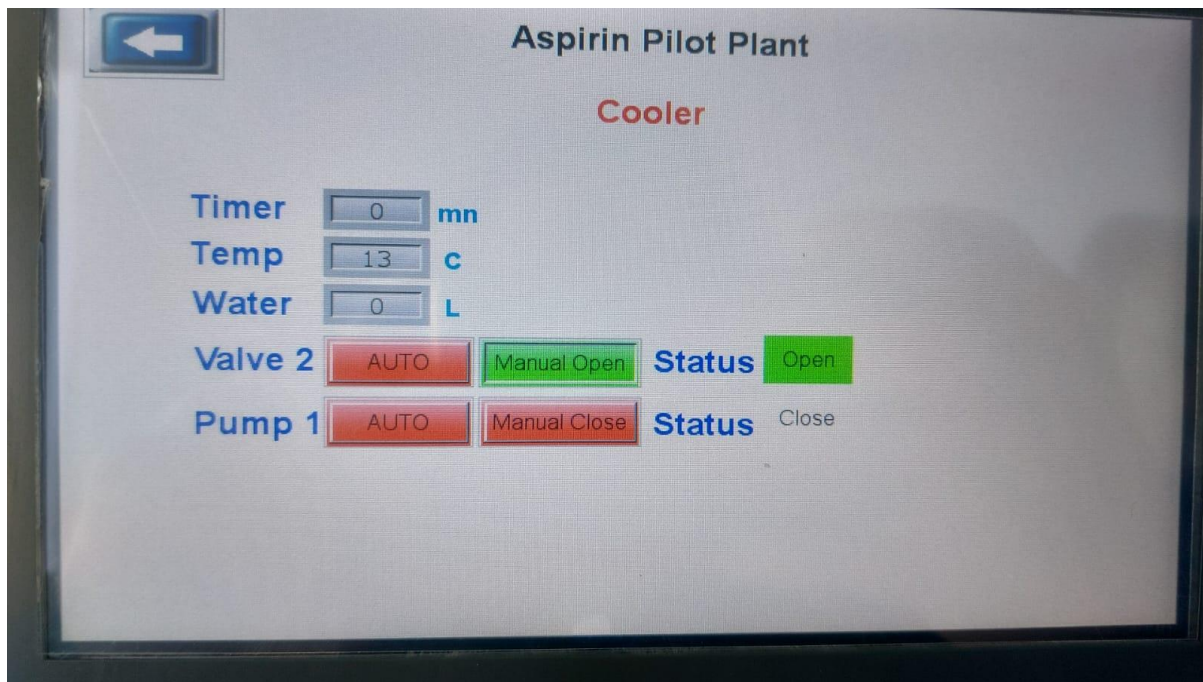


5. Turn ON manually valve 6 to transfer distilled water from Hot water tank (tank 2) to Cooler (tank 3) (PS: there's a flow sensor to determine the quantity needed of distilled water)

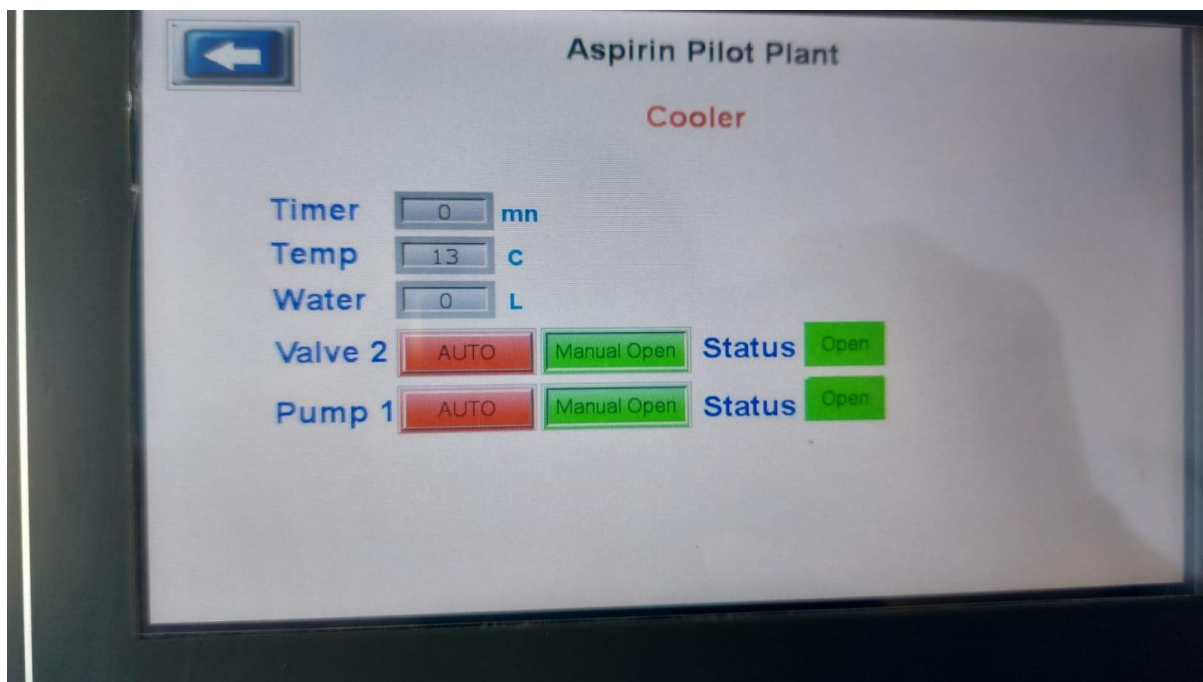


6. Turn ON manually the heater to warm up distilled water needed (PS: there's a temperature sensor to determine the temperature of DW required)

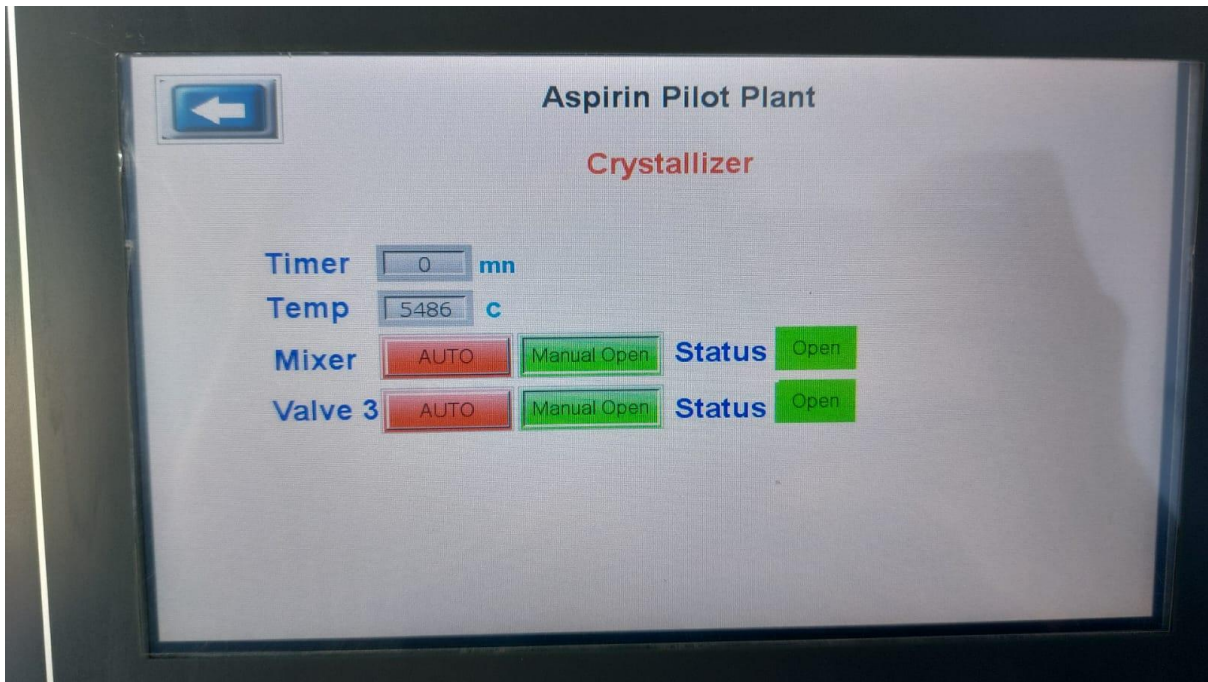
References:



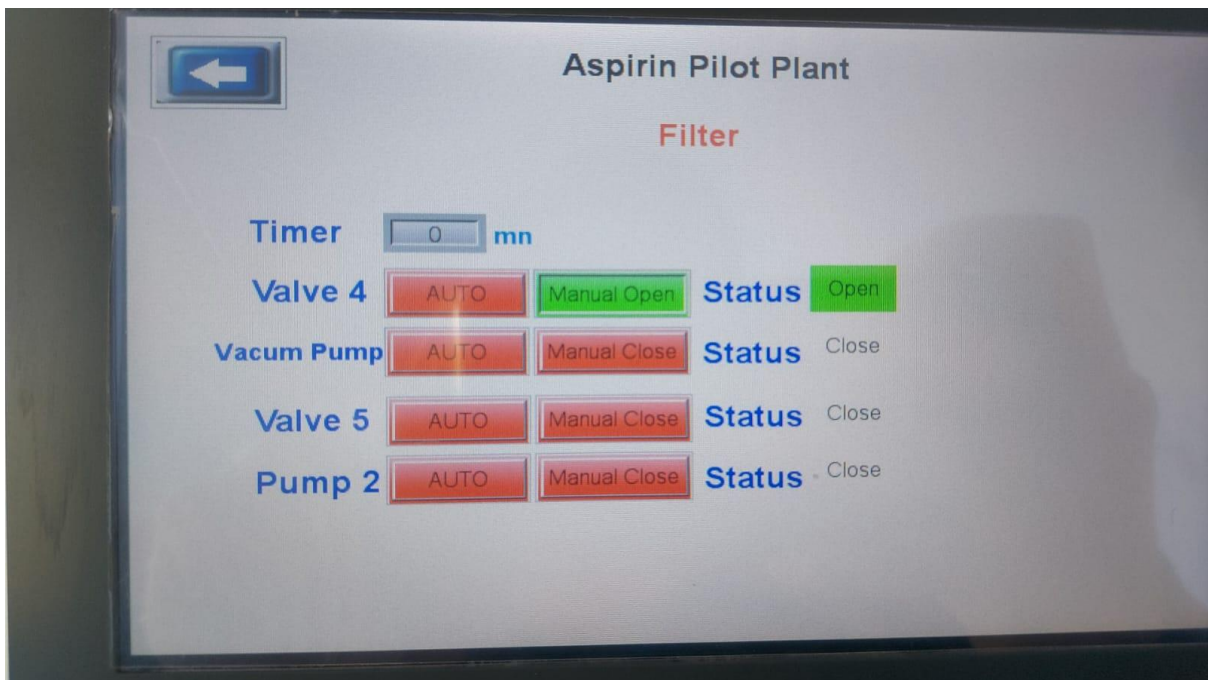
7. Turn ON manually valve 2 to transfer the mixture (Water) from the cooler (tank 3) to crystallizer (tank 4)



8. Turn ON manually pump 1 to transfer the mixture (Water) from the cooler (tank 3) to crystallizer (tank 4)

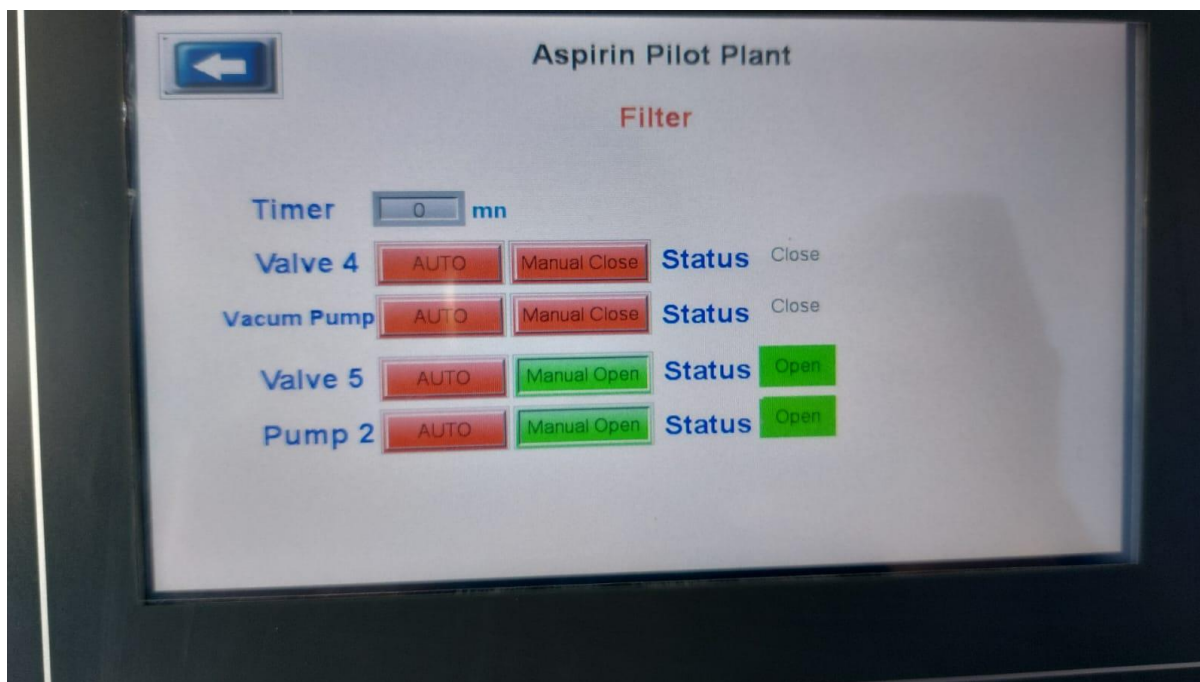


9. Turn ON manually the "Mixer" and valve 3 to mixing and transfer the mixture from the Crystallizer (tank 4) to the Filter (tank 5)



10. Turn ON the valve 4 to transfer the mixture (water) from the Filter (tank 5) to Waste tank (tank 6)

References:



11. Turn ON valve 5 and pump 2 to transfer the mixture (water) from Filter (tank 5) to crystallizer (tank 4) to recrystallization (**PURIFICATION STEP**)

5.4.3.3 005: Aspirin Pilot Plant flow sensor Test

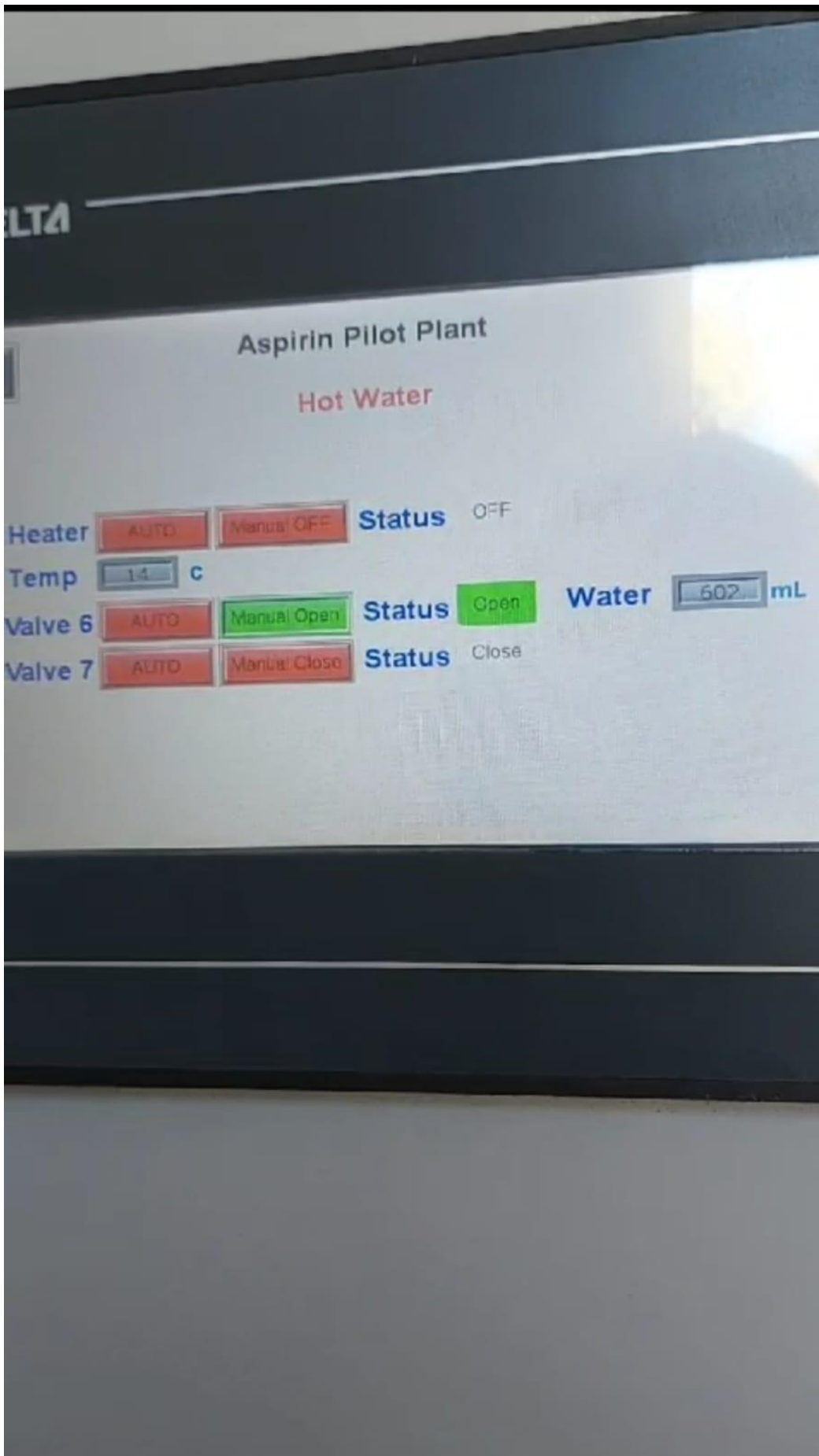
Step	Step Description	Results
Precondition	System is OFF	
TURNING ON the system	Turn ON the control panel	The system is ON
Open the valve 6 (tank 3 : HOT WATER)	Open the valve V6 to add water from HOT WATER TANK (tank 3) to the cooler (tank 2) (HOT WATER TANK---->HOT WATER TANK valve V6)	The water is in the cooler (tank 2) The flow sensor is marking the number of Litres going through
switching OFF the system	switch OFF the system	the system is OFF
Postcondition	system is OFF	

[005 video: video 2.1 \(test 1\) Flow sensor test in aspirin pilot plant 07/12/2023](#)



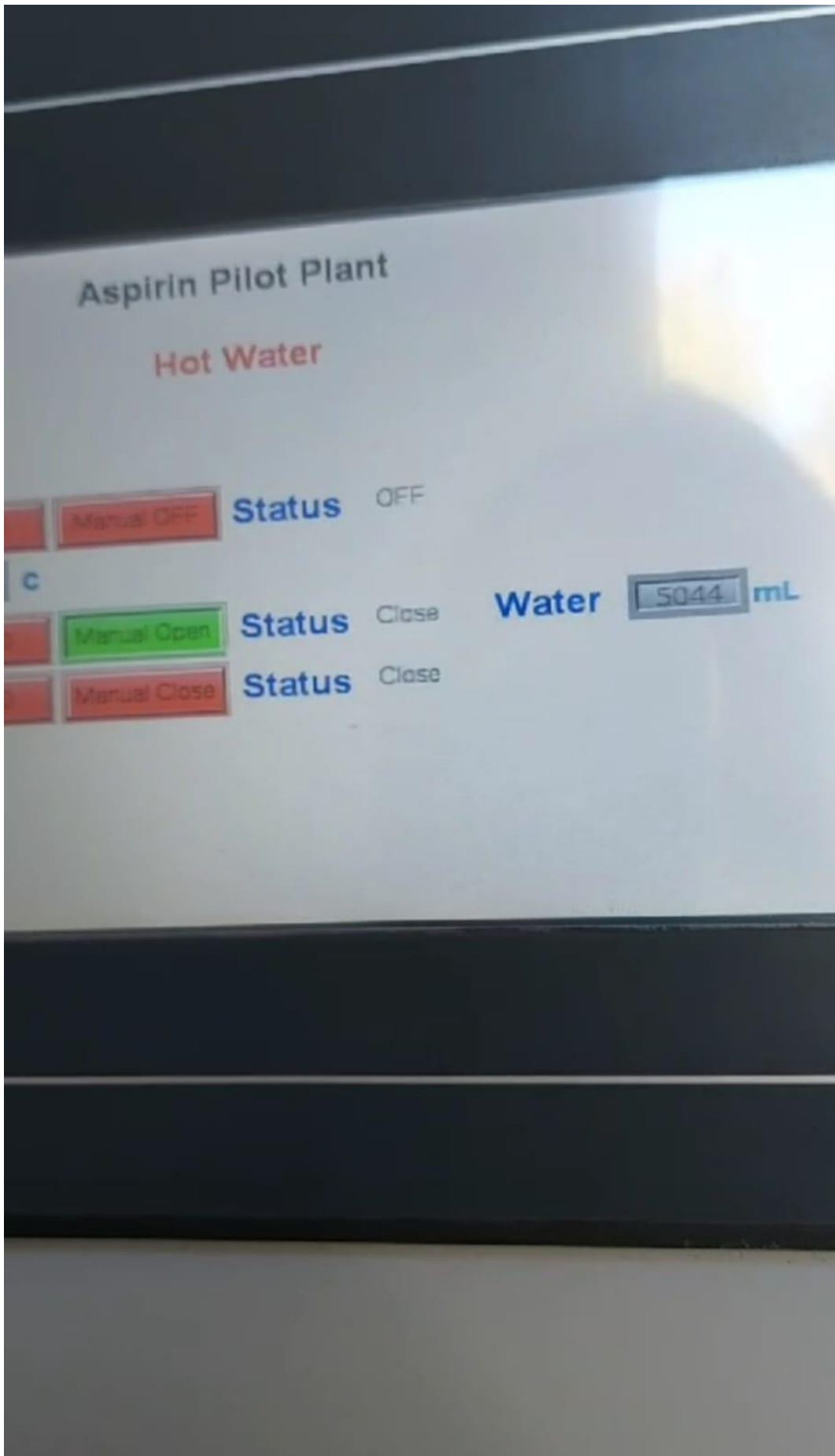
flow sensor test
control panel.mp4

References:



1. Turn ON the Valve V6 in HOT WATER TANK (tank 3) to transfer the Distilled Water and the flow sensor start counting quantity of DW in ml

References:



2. When the flow sensor marks 5L the Valve V6 will Turn OFF automatically.

References:

5.4.3.4 006: Aspirin Production System Test (Water test)

Step	Step Description	Results
Precondition	System is OFF	
TURNING ON the system	Turn ON the control panel	The system is ON
Switch on the mixer 1 (tank 1 : Reactor)	Turn on the mixer 1 from the control panel (Reactor--->Mixer ON)	Mixer 1 is ON and start Mixing the Water (Reactor)
Open the valve V1 (tank1 : Reactor)	Open the valve V1 to transfer the mixture from tank 1 (reactor) to tank 2 (cooler) (Reactor---->Reactor valve V1)	The Water is transferred to Cooler (tank 2)
Open the valve 6 (tank 3 : HOT WATER)	Open the valve V6 to add water from HOT WATER TANK (tank 3) to the cooler (tank 2) (HOT WATER TANK---->HOT WATER TANK valve V6)	-The water is in the cooler (tank 2) -The flow sensor is marking the number of Litres going through
Open the valve V2 (tank 2: Cooler)	Turn ON the pump 1 Open the valve V2 to transfer the Water from cooler (tank 2) to crystallizer (tank 4) (cooler---->cooler pump 1) (cooler---->cooler valve V2)	-The Water are transferred to the crystallizer (tank 4) -pump 1 pumping the water from tank 2 (cooler) to tank 4 (crystallizer)
Switch on the mixer 2 (tank 4 : Crystallizer)	Turn on the mixer from the control panel (Crystallizer--->Mixer ON)	Mixer 2 is ON and start Mixing the Water Crystallizer (tank 4)

Open the valve V3 (tank 4: Crystallizer)	Open the valve V3 to transfer the Water from crystallizer (tank 4) to the filter (tank 5) (crystallizer---->crystallizer valve V3)	The Water transferred to the filter (tank 5)
Open the valve V7 (TANK 3: HOT WATER)	Open the valve V7 to add water from HOT WATER TANK (tank 3) to filter tank(tank 5) (HOT WATER TANK---->HOT WATER Valve V7)	The Water transferred to filter tank (tank 5)
Open the valve V5 (tank 5: Filter)	Turn ON the pump 2 Open the valve V5 The Water is pumped from tank 5 to tank 4 for the second crystallization (filter---->filter Valve V5)	-Water transferred to the crystallizer (tank 4) -pump 2 pumping the water from tank 5(filter) to tank 4 (crystallizer)
switching OFF the system	switch OFF the system	the system is OFF
Postcondition	system is OFF	

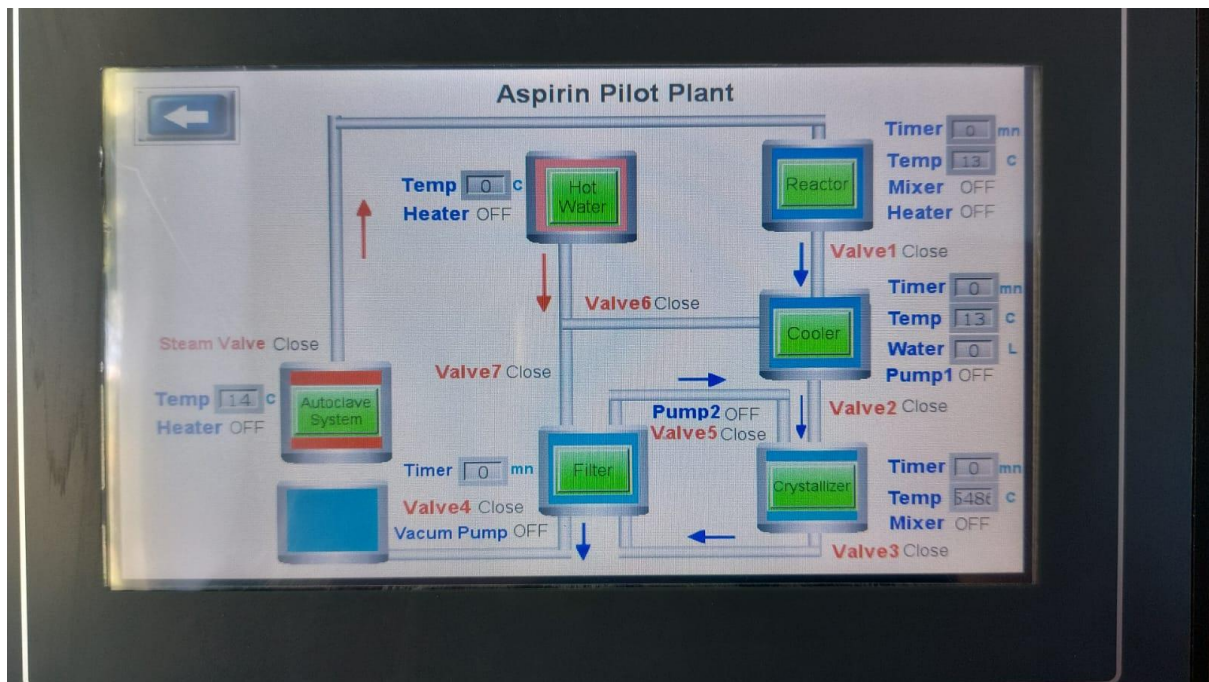
[video006: video 3.1 testing all mixers pumps and control system \(water only\) 14/12/2023](#)



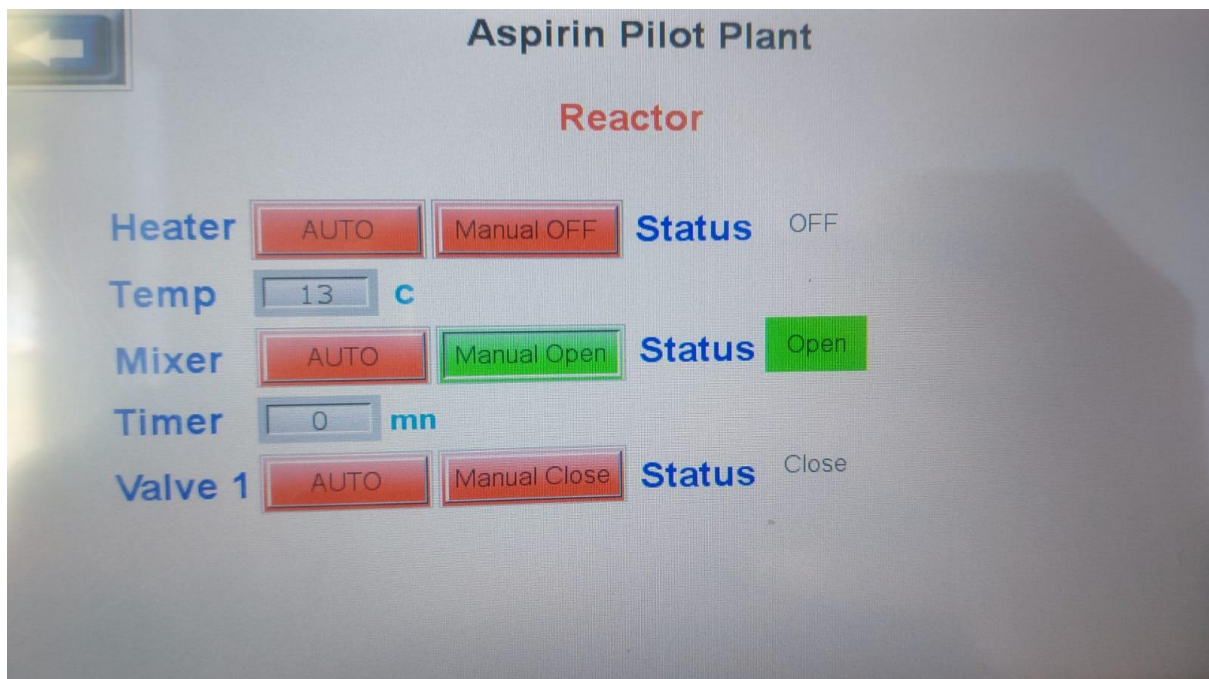
test video 3.1 system
test 14.12.23.mp4

Overview: Mixers, Valves and pumps are working well and this test was enough to prove this, so for the next step we should try the same test with the chemicals reagents

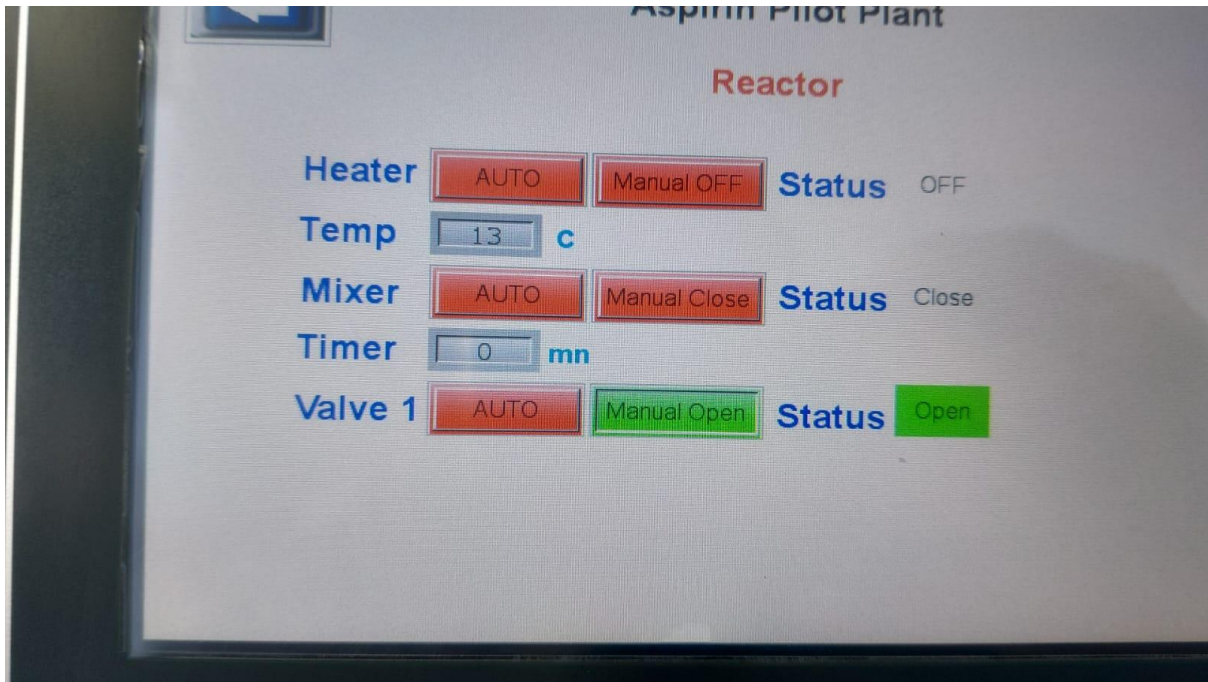
References:



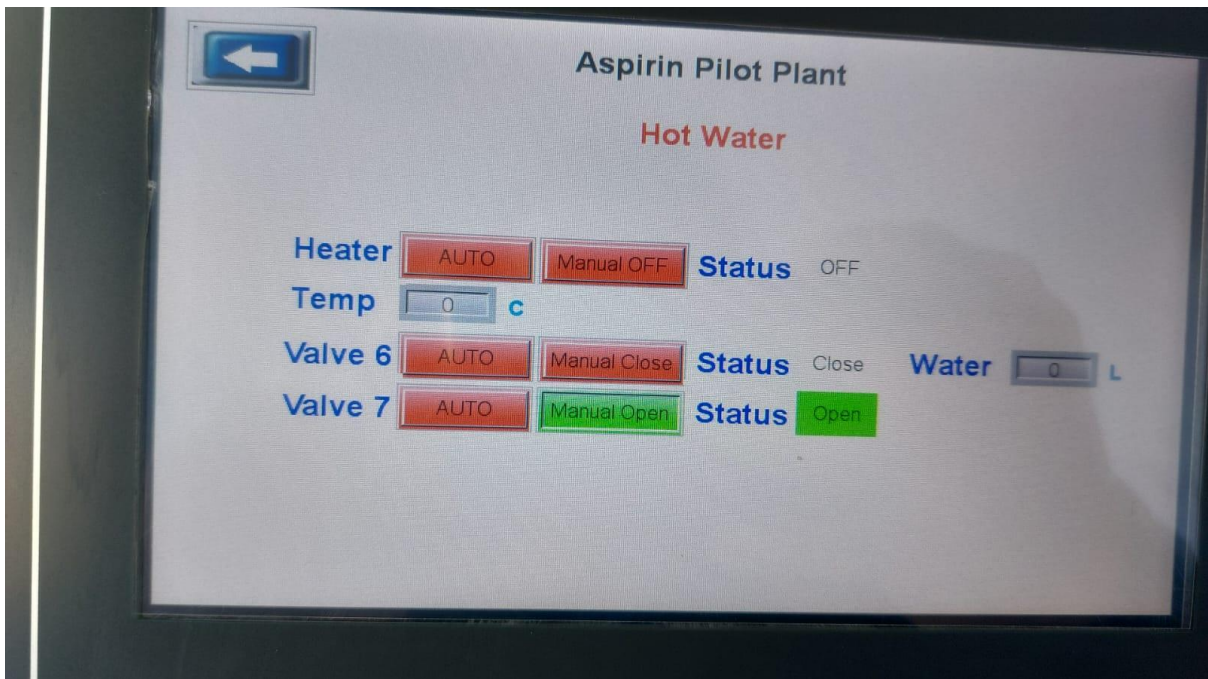
1. Overview of control panel system for Aspirin pilot plant



2. Turn ON the mixer by switching manually to mix the solution (PS: in this test we used water only)

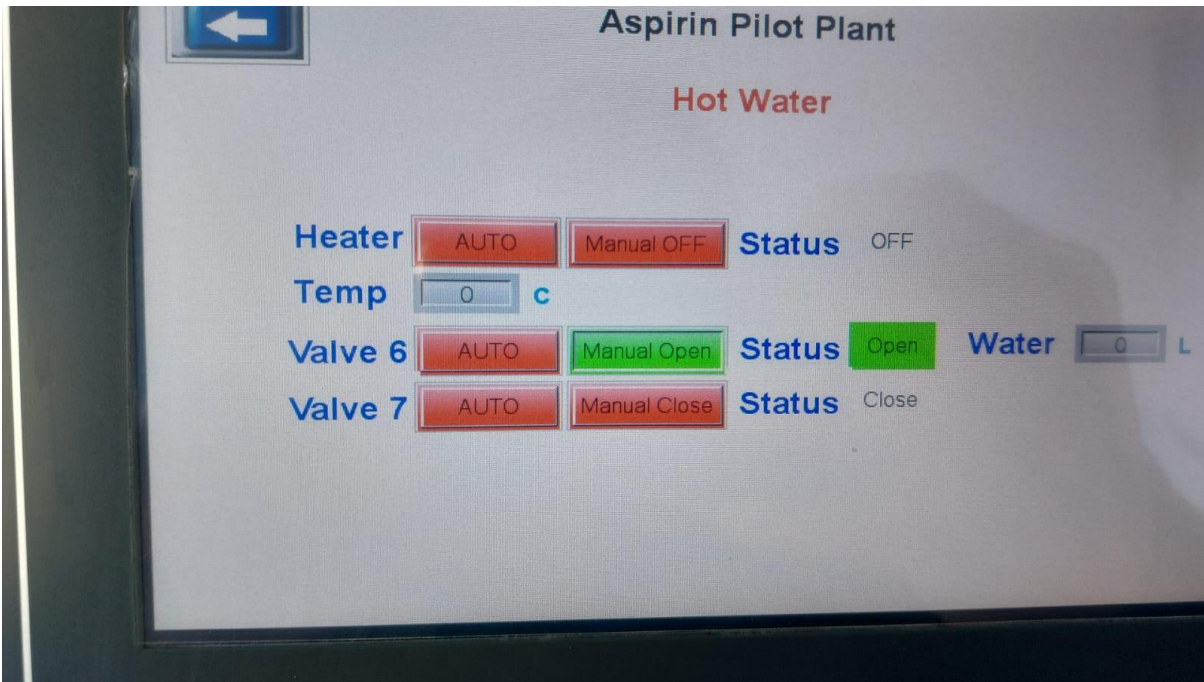


3. Turn ON manually valve 1 to transfer the solution to the Cooler (tank 3)

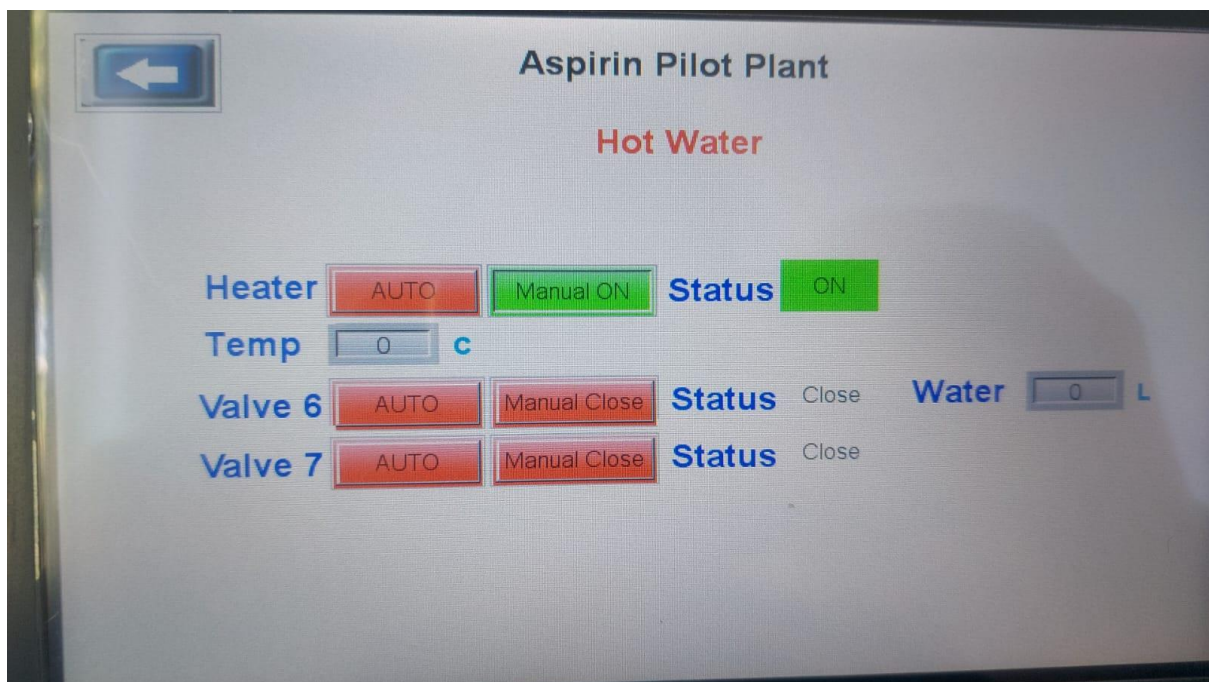


4. Turn ON manually valve 7 to transfer distilled water from Hot water tank (tank 2) to filter (tank 5)

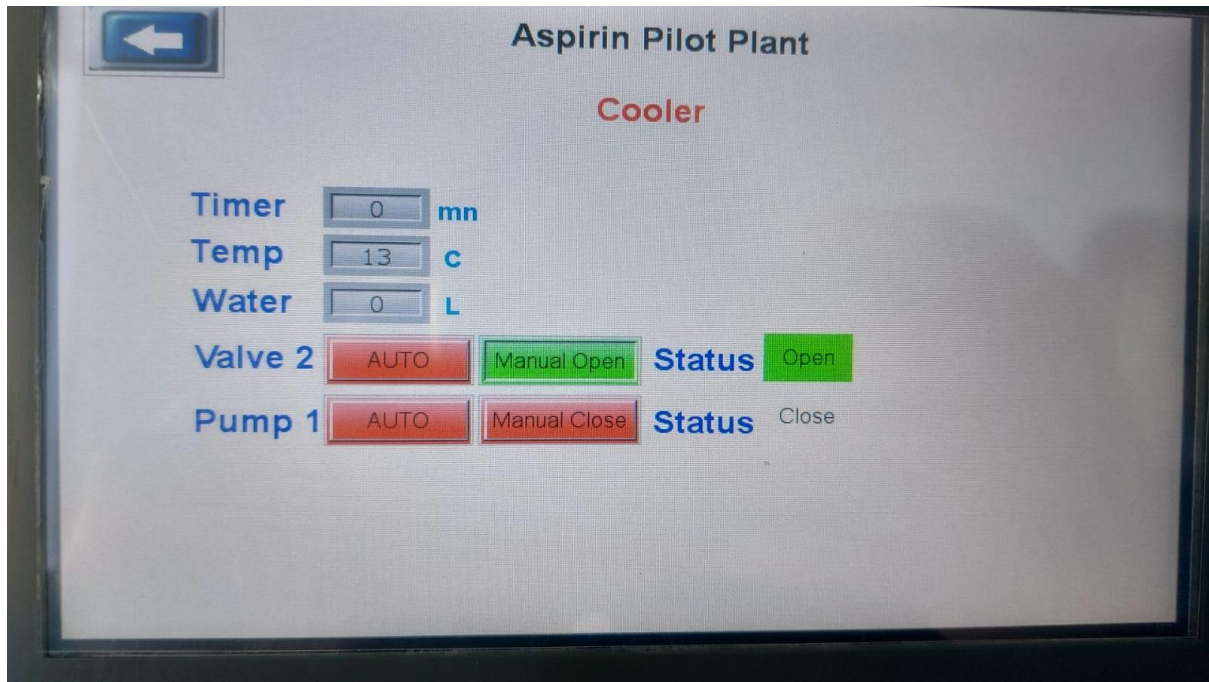
References:



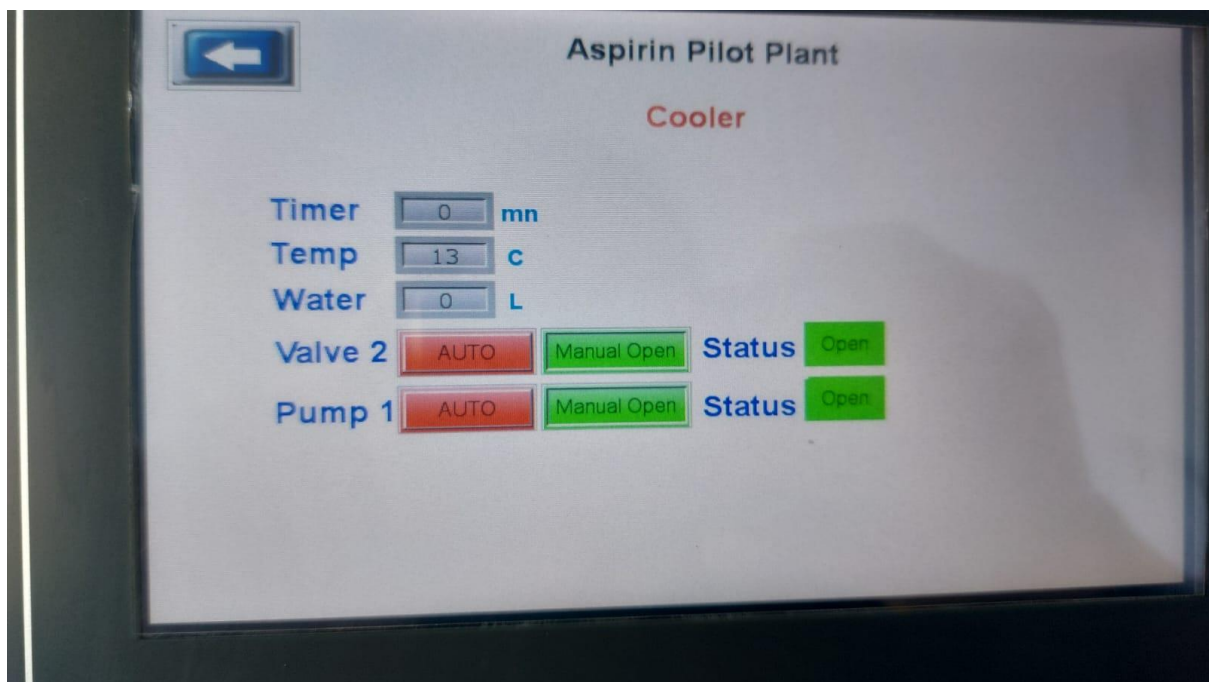
5. Turn ON manually valve 6 to transfer distilled water from Hot water tank (tank 2) to Cooler (tank 3) (PS: there's a flow sensor to determine the quantity needed of distilled water)



6. Turn ON manually the heater to warm up distilled water needed (PS: there's a temperature sensor to determine the temperature of DW required)

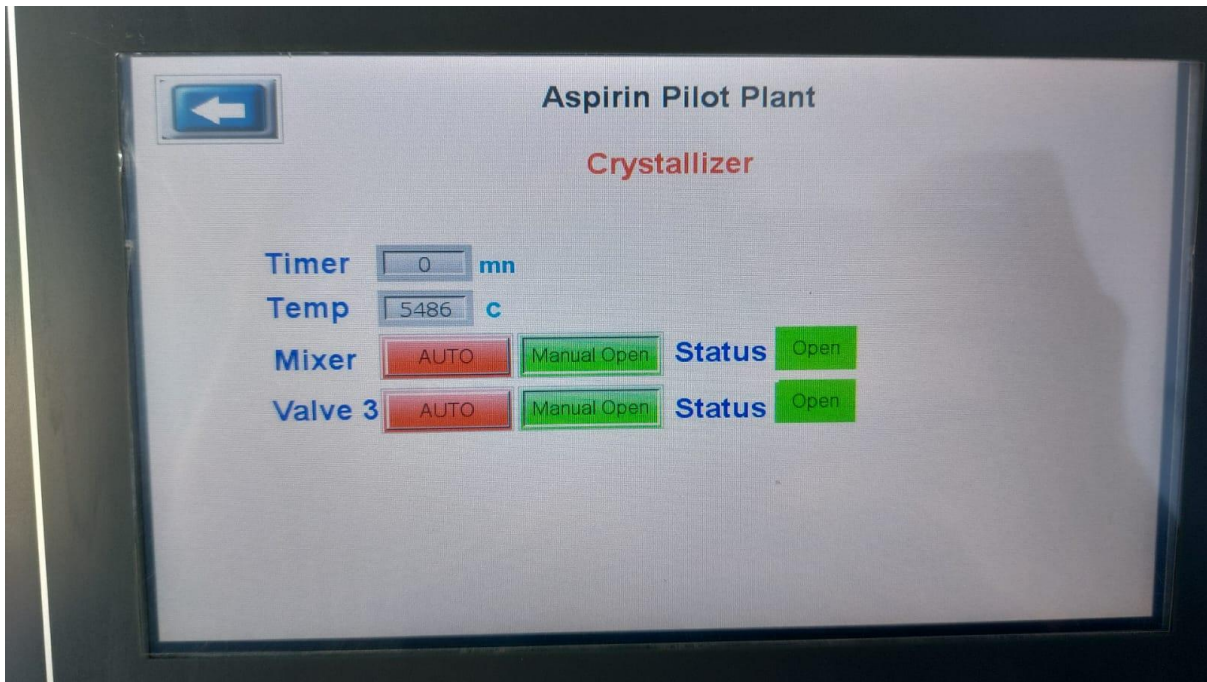


7. Turn ON manually valve 2 to transfer the mixture (Water) from the cooler (tank 3) to crystallizer (tank 4)

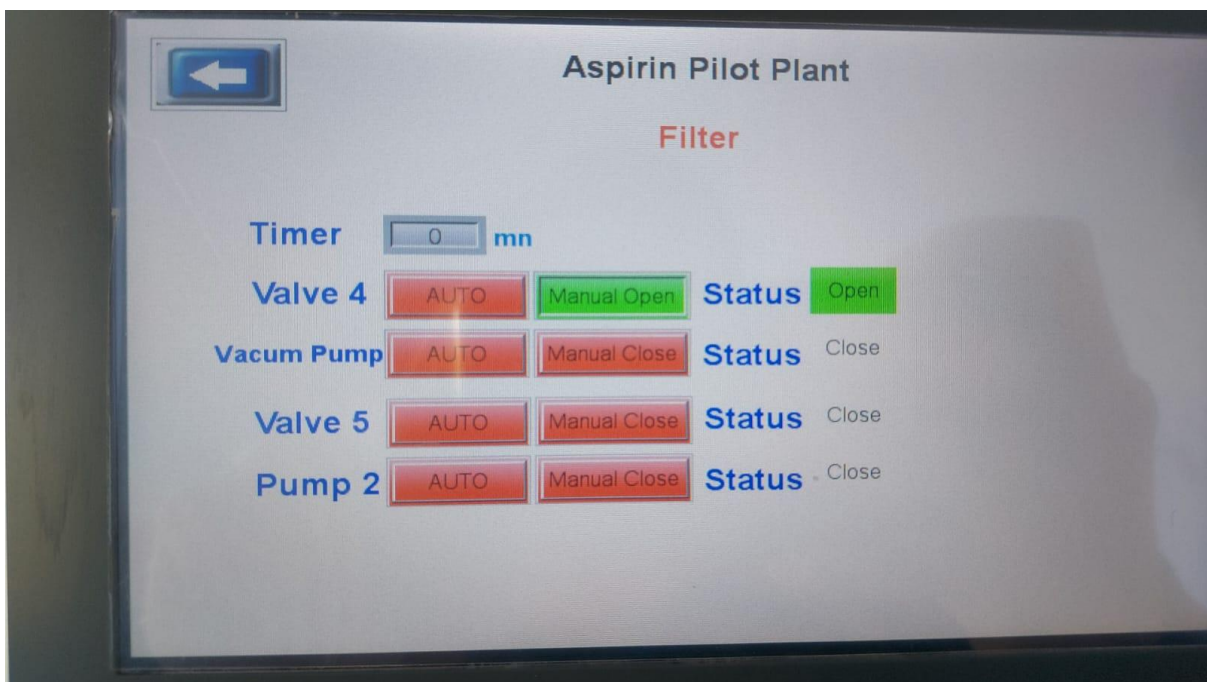


8. Turn ON manually pump 1 to transfer the mixture (Water) from the cooler (tank 3) to crystallizer (tank 4)

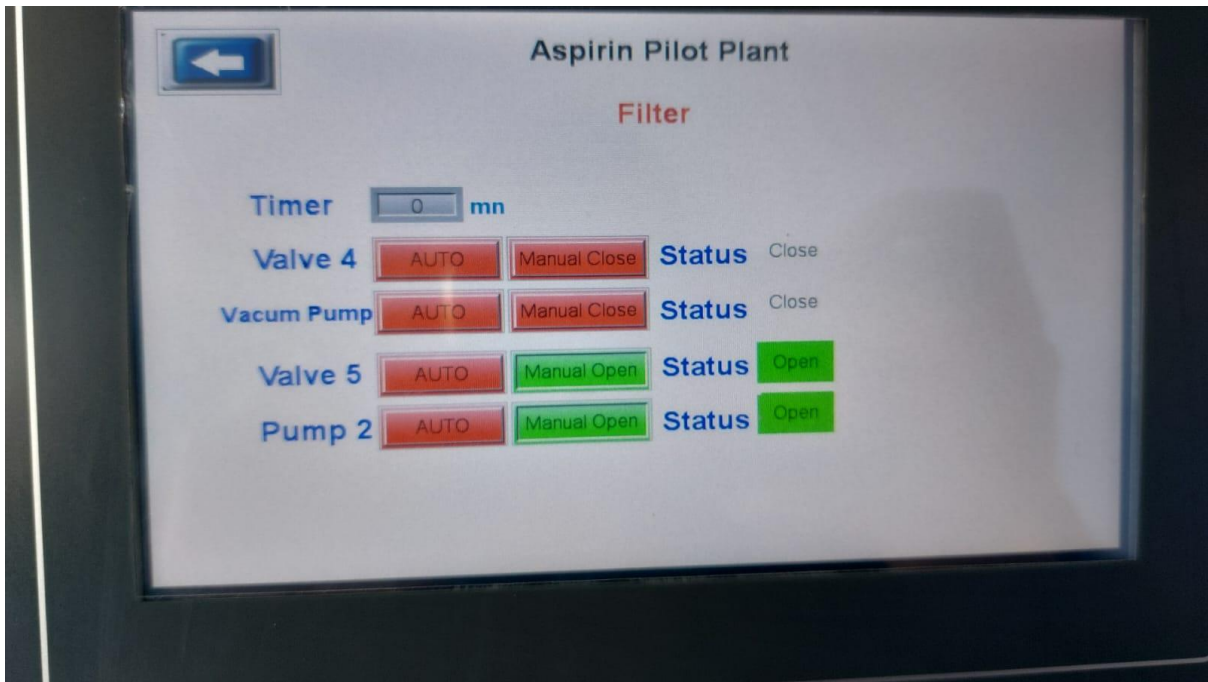
References:



9. Turn ON manually the "Mixer" and valve 3 to mixing and transfer the mixture from the Crystallizer (tank 4) to the Filter (tank 5)



10. Turn ON the valve 4 to transfer the mixture (water) from the Filter (tank 5) to Waste tank (tank 6)



11. Turn ON valve 5 and pump 2 to transfer the mixture (water) from Filter (tank 5) to crystallizer (tank 4) to recrystallization (PURIFICATION STEP)

References:

5.4.3.5 007: Aspirin Pilot Plant flow sensor Test

Step	Step Description	Results
Precondition	System is OFF	
TURNING ON the system	Turn ON the control panel	The system is ON
Open the valve 6 (tank 3 : HOT WATER)	Open the valve V6 to add water from HOT WATER TANK (tank 3) to the cooler (tank 2) (HOt WATER TANK---->HOT WATER TANK valve V6)	-The water is in the cooler (tank 2) -The flow sensor is marking the number of Litres going through (5L) and once we reach 5L the flow sensor will stop and valve 6 will close automatically.
switching OFF the system	switch OFF the system	the system is OFF
Postcondition	system is OFF	

[video 007: video 3.2 \(test 2\) flow sensor test in aspirin pilot plant 14/12/2023](#)



test video 3.2 flow sensor 14.12.23.mp4

ALTA

Aspirin Pilot Plant

Hot Water

Heater	<input type="button" value="AUTO"/>	<input type="button" value="Manual OFF"/>	Status	OFF			
Temp	<input type="text" value="14"/>	c					
Valve 6	<input type="button" value="AUTO"/>	<input type="button" value="Manual Open"/>	Status	<input type="button" value="Open"/>	Water	<input type="text" value="602"/>	mL
Valve 7	<input type="button" value="AUTO"/>	<input type="button" value="Manual Close"/>	Status	Close			

References:

1. Turn ON the Valve V6 in HOT WATER TANK (tank 3) to transfer the Distilled Water and the flow sensor start counting quantity of DW in ml

Aspirin Pilot Plant

Hot Water

Status OFF

Status Close **Water** mL

Status Close

References:

2. When the flow sensor marks 5L the Valve V6 will Turn OFF automatically.

5.4.3.6 008: ASPIRIN PILOT PLANT TEST (AUTOCLAVE TEST) ASPIRIN PILOT PLANT

Step	Step Description	Results
Precondition	System is OFF	
open/close the valves	Open all valves from the control panel and reclosed them after few minutes	Release the air from the system
Switch on the heater	Turn on the heater from the control panel	THE SYSTEM IS heating the water till reach 134°C
Sterilization the tank 1 (reactor)	Open the autoclave valve (autolave--->Autoclave valve)	-The steam is filling the reactor PS: there is leaking of steam in mixers rollings.
Sterilization the tank 2 (cooler)	Open the tank 1 (Reactor) valve V1 to sterilize tank 2 (cooler) (Reactor---->Reactor valve V1)	-No results
Sterilization the tank 3 (HOT WATER TANK)	Open the tank 3 (HOT WATER TANK) valve V6 to sterilize tank 3 (HOT WATER TANK---->HOT WATER TANK valve V6)	-No results
Sterilization the tank 4 (Crystallizer)	Open the tank 2 (cooler) valve V2 to sterilize tank 4 (crystallizer) (crystallizer---->crystallizer valve V3)	-No results
Sterilization the tank 5 (Filter)	Open the valve V3 to sterilize tank 4 (Crystallizer) to the tank 5 (Filter) (crystallizer---->crystallizer valve V3)	-No results

References:

Sterilization the tank 6 (Waste)	Open the valve V4 (Filter) to sterilize tank 6 (Waste) (Filter---->Filter valve V4)	-No results
Open the manual discharge valve	Open the discharge valve manually	-No results
CLose the valves	close ALL the valves from the control panel and the discharge valve manually	-No results
switching OFF the system	switch off the system	the system is OFF
Postcondition	system is OFF	

008video: video 3.3 (test 2) autoclave whole test 14/12/2023



test video 3.3
autoclave test 14.12.2

Overview: The leaking of the steam in mixers rolling make the test unsuccessful, we can't reach 2 bar in any tank due to leaking.

so the next step will be to focus this leaking and retest the aucolave system

5.5 Penicilline:

5.6 Cleaning/Tableting/Recycling Pilot plant

1. Cleaning process

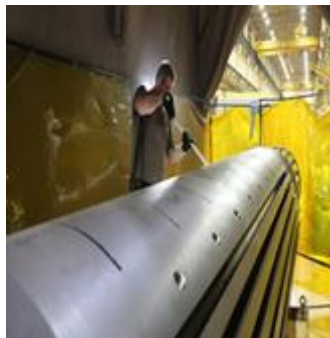
Tank cleaning is the most essential in the industries. There is usually a buildup of contamination on the reactor walls and on the agitator blade surface, which can be easily cleaned with different methods.

1. Hydro -blasting:

Hydro- blasting can be utilized to clean the insides of pipes, tanks, and process reactors.

Our process uses high pressure water jetting (15,000 psi to 50,000 psi) to remove scaling from the internal surfaces of pipe. This process is performed on steam piping prior to steam and air blowing of the steam lines to reduce the time, water, and fuel required to clean the steam piping.

The nozzle can be rotated 360° on a horizontal or vertical plane to form a crisscross pattern to thoroughly clean the tank and remove the stickiest residue



2. cryogenic cleaning

Dry ice cleaning consists of projecting particles of ice or dry ice, solid CO₂ at -78°C, by a flow of compressed air onto a surface to be cleaned. The combination of intense cold and mechanical shock causes the dirt to detach from its support.

- the particles of ice or dry ice are accelerated by a flow of compressed air.
- localized thermal shocks weaken the pollutants

References:

- cracks form and the pollutant cracks with the cold
- the micro- excavators strike the surface at a speed of 300 m/s and remove the loosened pollutants

3. chemical cleaning

Cleaning is achieved by physical action of high velocity flow, jet sprays, agitation and chemical action of cleaning agents enhanced by heat. While mechanical forces are necessary to remove gross soil and to ensure adequate penetration of cleaning solutions to all areas.

three steps: initial rinse, cleaning with detergent(s) and final rinse.

- Detergent: Alcon ox™ Liquinox™

Use to clean: pharmaceutical apparatus, industrial parts, pipes,tanks and reactor.

Surfaces cleaned: Corrosion inhibited formulation recommended for glass, metal, stainless steel.

Directions: Make a fresh 1% solution (2 1/2 tbsp per gal, 1 1/4 oz per gal, or 10 ml per L) in cold, warm or hot water. If available, use warm water. Use cold water for blood stains. For difficult soils, raise water temperature and use more detergent. Clean by soak, circulate, wipe...**RINSE THOROUGHLY**—preferably with running water.

- CIP process:
- **STEP 1: PRE-RINSE**

The pre-rinse is a very important step in the CIP

The pre-rinse cycle:

- Wets the interior surface of the lines and tank
- Removes most of the remaining residue
- Dissolves sugars and partially melts fats
- Provides a non-chemical pressure test of the CIP flow path

Use potable plant water, de-ionized water (DI).

A Turbidity Sensor may be used to verify that the pre-rinse effectively removes all solids.

-

- **STEP 2: CAUSTIC WASH – (140° – 185° F)**

Caustic washes soften fats, making them easier to remove. Also known as caustic soda, sodium hydroxide or NaOH.

Caustic is typically used as the main detergent in most CIP wash cycles. A non-foaming formulation can help reduce pump cavitation and increase efficiency.

Water Saving Tip: In many cases, the caustic wash can be returned to its tank and re-used multiple times, which significantly reduces water, chemical, and energy costs over a single tank system.

- **STEP 3: INTERMEDIATE RINSE**

Fresh water flushes out residual traces of detergent remaining from the caustic wash.

- Level Transmitters and Probes monitor tank levels of wash and rinse tanks.
- Flow Transmitters ensure optimum flow for spray devices to precisely control wash and rinse steps.
- Conductivity Transmitters ensure chemical levels are hitting predetermined set point.
- **STEP 4: FINAL RINSE**

Rinse with either DI, RO, or city water to flush residual cleaning agents.

Note! the final rinse water may be recovered and reused as the pre-rinse solution for the next cleaning cycle.

- **STEP 5. SANITIZING RINSE**

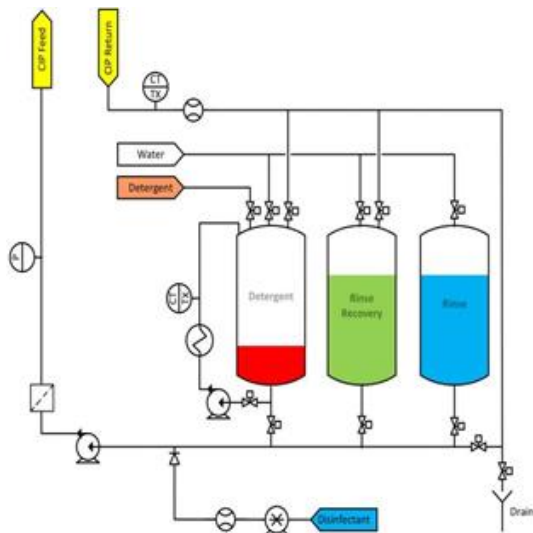
May be required to help kill microorganisms before starting the next production run. The PAA solution:

- Is a strong disinfectant even at low temperatures.
- Rinses away while leaving little or no chlorine residue to corrode stainless steel.

References:

- Is effective against all microorganisms including spoilage organisms, pathogens and bacterial spores.
- It has also proven to be eco-friendlier in the wastewater stream.
- Has a strong, pungent odor so it should only be used in well-ventilated areas.
- Warning: Sanitizers reduce bacterial growth but don't completely kill all pathogens in the system; since it is the last step in the cleaning process, re-circulating the sanitizing solution could run the risk of spreading any leftover contamination that might be present. Sanitizers can also be sensitive to high temperatures and can lose their effectiveness fairly rapidly once they are in solution.

Note! Peracetic acid should be safe with stainless steel



finally we decide to use **hydro-blasting** method by dividing this method into three steps : pre-rinse addition of detergent, and final rinse.

2. Aspirin tableting process

Tableting aspirin is beneficial because it makes the medication easier to swallow and ensures a consistent dose. It also helps to prevent the medication from breaking down too quickly, allowing it to be stored for longer periods of time.

-First method:

Procedure:

A stable aspirin tablet may be prepared under the conditions where RH (Relative humidity is amount of water present in an air particle over can be measured by hygrometer) is maintained below 30 %, by the process comprising steps of:

1. Sifting aspirin, and microcrystalline cellulose or corn starch.
2. Blending the material of step 1.
3. Sifting the pregelatinized (Examples of binders include pregelatinized starch: polyvinyl pyrrolidone) and Silica colloidal anhydrous (Example of lubricant and glidant) and adding to material of step 2.
4. Sifting the stearic acid and adding to material of step 3 and blending.
5. Compressing the blend of step 4, The above blend was compressed using approved punches and dies.
6. Preparing the enteric coating dispersion by adding and mixing talc hydrated magnesium silicate (apply a safe barrier against contamination as a glidant to improve powder flow in tablet compression), meth acrylic acid-ethyl acrylate copolymer (1:1), triethylcitrate and simethicone emulsion in water
7. Spraying the dispersion onto the tablet.

Formula:

Ingredients	Amount (mg)	Role
Aspirin	75.00	Active ingredient
Corn starch	24.20	Disintegrant
Starch Pregelatinized	7.70	Binder
Silica Colloidal Anhydrous	2.20	Anticaking agent, emulsion stabilizer, glidant
Stearic Acid	0.90	Aspirin against degradation
Talc	3.33	Glidant
Meth acrylic Acid-Ethyl Acrylate Copolymer (1:1)	10.50 (As dry substance)	Tablet binder, tablet coating agent

References:

Triethyl Citrate	1.05	It allows a slower release of the contents of tablets
Simethicone Emulsion	0.12 (As dry substance)	Emulsion
Purified Water	q.s (Quantum satis=the amount which is enough)	used in Preparation of enteric coating

-Second method

Requirement:

- Chemicals: Aspirin, starch, polyvinyl pyrrolidone, ethanol, magnesium stearate, talc.
- Glass wares: measuring cylinder, beaker, mortar and pestle, granulating sieve...
- Equipment: tray dryer, tablet press.

Procedure:

1. Weight and pass aspirin and starch powder through 60#sieve (size with a 0.0098" (250 μ m) nominal sieve opening with a typical wire diameter of 0.16mm).



2. Mix aspirin and starch uniformly in mortar and pestle



3. Prepare 10% PVP solution in ethanol and stir until it becomes clear



4. Add PVP solution dropwise in mortar to get cohesive mass



5. Screen prepared cohesive mass through 10#granulating sieve (a medium size U.S. Standard mesh size with a 0.0787" (2mm) nominal sieve opening with a typical wire diameter of 0.9mm) and collect it on granulating tray

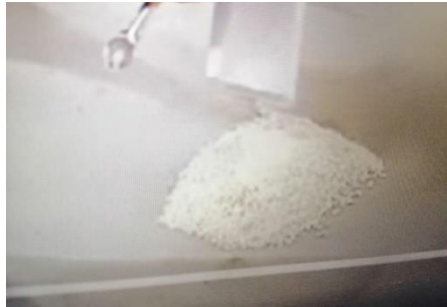


6. Dry granules in tray dryer at 50C° for 30min

References:



- Blend granules with remaining ingredients (talc and magnesium stearate) using polybag



- Store prepared granules in well closed and labelled container

Note: **Corn starch** is most suited as a vehicle for tablet compression in the pharmaceutical industry. **PVP** improving the bioavailability and stability of drugs, improving the physic mechanical properties of preparations, adjusting the release rate of drugs.

Formula:

Sr. No.	Ingredient	Quantity Given (1 tablet)	Quantity taken (50 tablets)	Role
1	Aspirin	300 mg	1.5 g	Active Ingredient
2	Polyvinyl pyrrolidone (10 % w/v solution in ethanol)	q.s.	q.s.	Binder (ethanol as granulating liquid)
3	Starch	15 mg	0.75 g	Disintegrant
4	Talc	10 mg	0.5 g	Glidant
5	Magnesium Stearate	10 mg	0.5 g	Lubricant

Note: this table contains a mistac in the quantity taken of aspirin (50 tablets) 15g instead of 1.5 g

To watch a video explaining click on this link :

https://www.youtube.com/watch?v=r_3bSQd2xv0&t=1453s

3. Evaluation test

A.Evaluation of granules

The Prepared Tablet is Evaluated in terms of bulk density, tapped density, the angle of repose, Carr's Index and, hardness test, weight variation test, friability test and in vitro study. The result associated with optimized batch is good satisfactory and having better drug release kinetic.

We will have used the method and the into evaluated granules: bulk density, tapped density, the angle of repose and Hausner's ratio.

A-A volume of powder is filled into a graduated glass cylinder and repeatedly tapped for a known duration. the volume of powder after tapping is measure.

1.**Bulk density**=weight /bulk volume (Bulk density: This includes the volume of solid fraction of particles and intra- and interparticulate volumes, Bulk density is defined as the mass of the many particles of the material divided by the total volume they occupy).

2.**tapped density** =weight / tapped volume (The tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample)

The tap density tester is ideal for measuring the tapped density of powders:

References:



B. The Hausner's ratio is a number that is correlated to the flow ability of a powder or granular material. The Hausner ratio is used in a wide variety of industries as an indication of the flow ability of a powder.

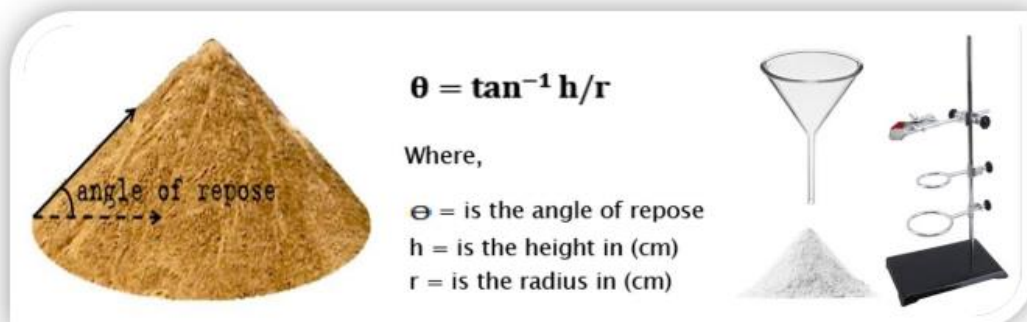
Hausner's ratio = Tapped density / bulk density

A Hausner Index greater than 1.2 is considered an indication of poor flow but good compressibility and good cohesion.

C. Angle of repose is a parameter commonly used for the evaluation of interparticle force, Angle of rest is defined as the angle that an inclined plane makes with the horizontal when a body placed on it just starts sliding.

How can be calculate angle of repose?

The simplest method for the determination of the angle of repose is the "poured" angle. A funnel with a wide outlet is affixed at a distance of 10 cm above a piece of paper is placed directly beneath the funnel. Powder is added while the funnel is closed. The contents flow through and collect on the paper. The diameter of the cone (D) and two opposite sides (l1 + l2) are measured with rulers. The angle of repose (θ) is calculated from the equation $\text{arc cos } [D/(l1 + l2)]$. The relationship between flow properties and angle of repose has been established. !!When the angle of repose is less than 25 degrees, the flow is said to be excellent; on the other hand, if the angle of repose is more than 40 degrees, the flow is considered to be poor.



B. Evaluation of tablets

Disintegration test for tablets:

The disintegration test is used to show how quickly the tablet breaks down into smaller particles, allowing for a greater surface area and availability of the drug when taken by a patient.

To carry out a disintegration test for tablets, we use a basket which holds 1 to 6 tablets. This is then raised and lowered into a beaker of water, which is used to simulate conditions in the stomach at 37°C. If the tablets or capsules float, perforated plastic disks are placed on the top of the tablets to keep them under the water level. The tablet disintegration time is taken when no residue is left in the mesh.



cling

4. Recy

The production of aspirin typically generates waste byproducts which can be harmful to the environment if not disposed of properly. Recycling of aspirin production waste can be done through various methods such as:

I. Recovery of salicylic acid: Salicylic acid can be recovered from the waste generated during aspirin production through a process called acidification and crystallization. Here are the basic steps involved in the process:

1. Acidification: The waste solution containing salicylic acid is first acidified with a strong mineral acid such as hydrochloric acid or sulfuric acid to convert the salicylic acid to its protonated form. The acidification is done by slowly adding the mineral acid to the waste solution while stirring until the pH of the solution reaches around 2. The protonation of salicylic acid is described by the following equation:

References:

$C_7H_6O_3 + H_3O^+ \rightarrow C_7H_7O_3^+ + H_2O$. where $C_7H_6O_3$ represents salicylic acid and H_3O^+ represents the hydronium ion.

For example, if there is 1 gram of excess salicylic acid in the crude aspirin mixture, then approximately 1.5 to 2 grams of sulfuric acid would be added to react with the excess salicylic acid.

It's important to note that the exact quantities used can vary depending on the specific process and the conditions under which the acidification is carried out. Additionally, the acidification step must be carefully controlled to avoid the formation of undesirable byproducts or the degradation of the salicylic acid.

2.Extraction: The protonated salicylic acid is then extracted from the waste solution using an organic solvent such as ethyl acetate, dichloromethane, or toluene. The organic solvent is added to the acidified solution and the mixture is shaken or stirred for a few minutes to allow for the extraction of the protonated salicylic acid into the organic layer. The organic layer is then separated from the aqueous layer using a separating funnel. The quantities used in the extraction process (such as the amount of organic solvent or washing solution used) can vary depending on the specific process and the amount of crude aspirin being processed.

3.Recrystallization: The organic layer containing the protonated salicylic acid is then evaporated to dryness to obtain the crude salicylic acid. The crude salicylic acid is then dissolved in a suitable solvent such as ethanol or methanol and heated to dissolve the salicylic acid completely. The solution is then allowed to cool slowly to room temperature, causing the salicylic acid to recrystallize from the solution. The crystals are then filtered, washed with cold solvent to remove any impurities, and dried to obtain pure salicylic acid. the general amount used is about 3-5 times the mass of the salicylic acid being recrystallized. For example, if you have 1 gram of salicylic acid, you would use approximately 3-5 mL of ethanol as the solvent for the recrystallization step.

Once the salicylic acid has been recovered and purified, it can be reused in the production of aspirin or other products that require salicylic acid as a starting material.



II. Recycling of acetic acid:

- Reuse of acetic acid: The acetic acid produced during aspirin production can be purified and reused in subsequent batches of aspirin production. This reduces the amount of waste generated and saves on production costs,

Acetic acid is commonly used in the synthesis of aspirin as a catalyst to help facilitate the reaction between salicylic acid and acetic anhydride.

Extraction of acetic acid: in addition, acetic acid can be recovered by distillation or by extraction with an appropriate solvent such as ether or dichloromethane. Once recovered and purified, acetic acid can be reused in other chemical reactions, thus reducing costs and minimizing waste from aspirin production.

Add the solvent: A small amount of solvent is added to the mixture of water and acetic acid. **Mix thoroughly:** The mixture is mixed thoroughly to ensure that the solvent and water/acetic acid mixture are fully in contact with each other. **Allow to separate:** The mixture is then allowed to separate into two layers - the aqueous layer (containing water and acetic acid) and the organic layer (containing the solvent).

Collect the organic layer: The organic layer is carefully separated from the aqueous layer and collected in a separate container.

Repeat the extraction: The extraction process may be repeated multiple times to increase the yield of acetic acid.

Recover the acetic acid: The solvent is then evaporated from the organic layer to recover the acetic acid.

- **Neutralization:** The waste acetic acid can be neutralized using an appropriate base such as sodium hydroxide or calcium hydroxide. This results in the formation of a salt that can be disposed of safely. The neutralization reaction of acetic acid, which is a weak acid, with a strong base, such as sodium hydroxide (NaOH), can be represented by the following chemical equation:



To determine the exact amounts needed for a given neutralization reaction, one must know the concentrations of the reactants and use stoichiometry calculations to determine the amounts needed. For example: if one wishes to neutralize 10 mL of a 0.1 M acetic acid solution with 0.2 M sodium hydroxide, one would need to add 20 mL of the NaOH solution to the acetic acid while gently shaking until neutralization is complete.

III. Recycling of acetic anhydride

In the case of extracting acetic anhydride from the waste products of aspirin production, a suitable solvent is needed that will selectively dissolve the acetic anhydride. One possible solvent

References:

for this purpose is dichloromethane (also known as methylene chloride). Here are the steps for extracting acetic anhydride from the waste:

- Add the waste mixture to a separator funnel.
- Add enough dichloromethane to the separator funnel so that the mixture is fully covered.
- Close the funnel and shake it gently to allow the solvent to mix with the waste mixture.
- Wait for the mixture to settle, with the denser layer at the bottom and the lighter layer (containing the acetic anhydride) on top.
- Slowly drain off the lighter layer (the dichloromethane layer containing the acetic anhydride) into a clean container.
- Discard the heavier layer (the aqueous layer containing the waste products).

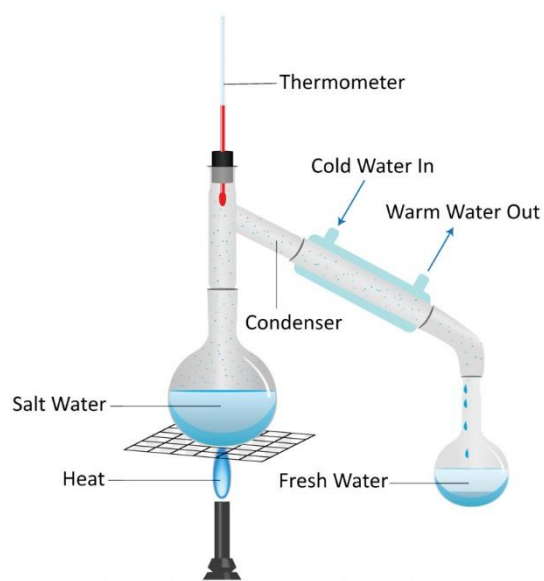
The extracted acetic anhydride can then be further purified using methods such as distillation or recrystallization.

Recrystallization of acetic anhydride can be done by following the steps:

- Dissolve acetic anhydride in an appropriate solvent such as ethanol or acetone. The amount of solvent used will depend on the amount of acetic anhydride to be recrystallized.
- Heat the solution gently until all the acetic anhydride is dissolved.
- Slowly add the recrystallization solvent, usually water or acetone, to the hot solution, stirring constantly, until the solution is saturated and crystals begin to form.
- Allow the solution to cool slowly, preferably to room temperature, to allow crystals to form.
- Filter the crystals formed using a Büchner funnel and wash with the recrystallization solvent.
- Dry the crystals in the open air or in a low temperature oven

It is important to note that the recrystallization can be carried out several times to improve the purity of the crystals.

IV. Recycling of water: Water is an important component in the production of aspirin, and it is often used in large quantities. The water used can be treated and recycled for use in subsequent batches of aspirin production. evaporation and condensation: This process involves evaporating the waste water to remove impurities and then condensing the water vapor to produce purified water. The purified water can then be reused in the manufacturing process.



note :

Extraction of salicylic acid: It is generally extracted first because it is the main compound in the reaction mixture of the synthesis of aspirin.

Extraction of acetic acid: If you need to recover the acetic acid, you can do it after extracting the salicylic acid because it is present in lesser quantity in the reaction mixture.

Extraction of acetic anhydride: If you want to recover acetic anhydride from waste from aspirin production, this can be done after recovering acetic acid, since acetic anhydride is synthesized from acetic acid. However, please note that recovering acetic anhydride from waste can be more complicated than the first two extractions.

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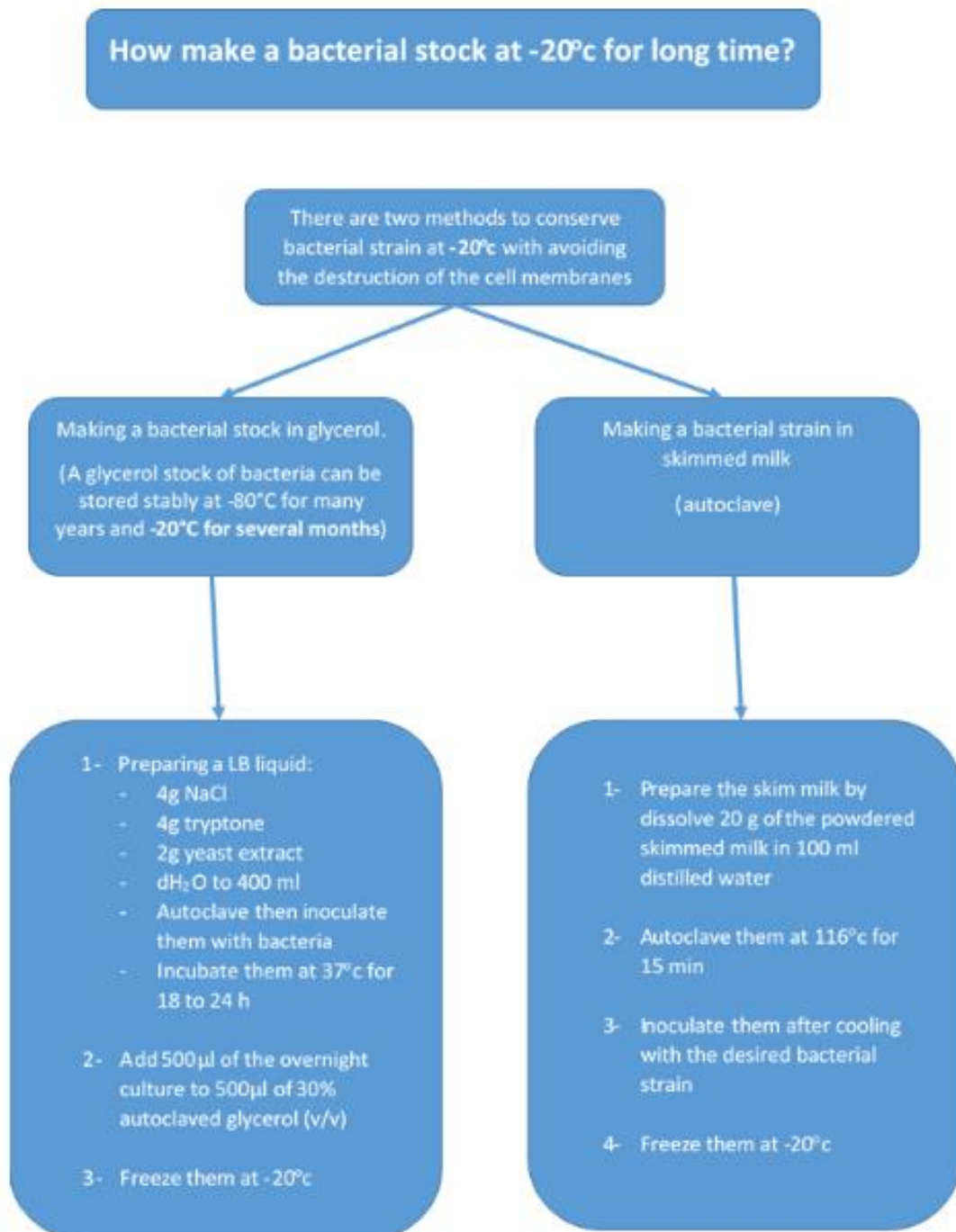
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7 Annex:

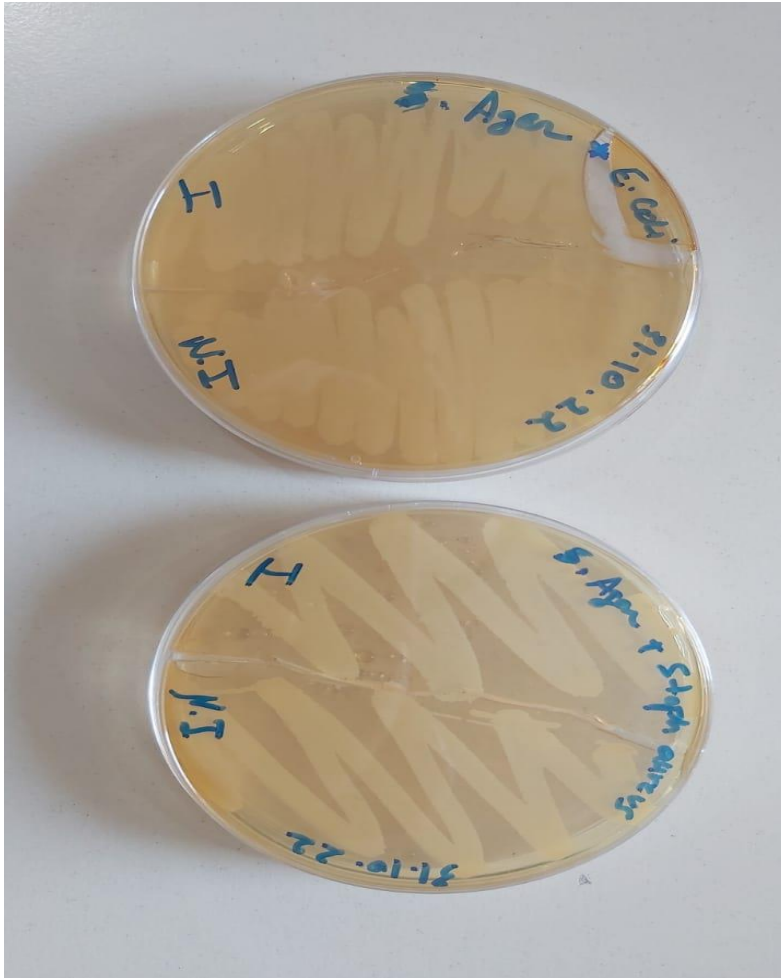
1.1 The most important solutions in biology lab

1.1.1 Making bacterial stock solution:27-10-2022



Realisation:28-10-2022

The strain will be fresh to be inoculated and then frozen so E.coli and S.aureus must be renewed 24 hour before and then inoculated in skim milk before freezing at -20°C



1.1.2 Solution preparation

1.1.2.1 NaOH(1M):

- Fill the flask half way with distilled water
- Add 40g of NaOH powder
- Add water to reach the 1L mark
- finally mix it to obtain NaOH solution (1M).

1.1.2.2 **H₂SO₄(1M):**

-Fill the flask half way with distilled water

Add 56 ml of H₂SO₄

-add water to reach the 1L mark

-Finally mix it to obtain H₂SO₄ solution (1M).

1.1.2.3 **NaCl 0.9%:**

-We put 0.9g NaCl in 100 ml distilled water

-Put it in Autoclave for microbial usage.

1.1.2.4 **Bleach 1%:**

-We took 1ml of bleach solution

-Put it in 100ml water graduated cylinder

-Mix it to obtain Bleach solution 1%.

1.1.2.5 **Muller Hinton agar:**

-Measure 5.7g of Muller Hinton powder

-Put it in 150 ml of distilled water

-Mix them on magnetic stirrer with heating (to reach boiling point)

-Put it in Autoclave (1h) for microbial usage.

1.1.2.6 **Standard agar:**

-Measure 5.55g of standard agar powder

Annex:

-Put it in 150 ml of distilled water

-Mix them on magnetic stirrer with heating (to reach boiling point)

-Put it in Autoclave (1h) for microbial usage.

1.1.2.7 Broth:

-Measure 5g peptone, 1g tryptone, 1g glucose, 1g yeast extract and 2.5g sodium chloride

-Put it in 500ml of distilled water

-Mix them on magnetic stirrer with heating (to reach boiling point)

-Put it in Autoclave (1h) for microbial usage.

1.1.2.8 Phosphate buffer (0.1M, pH:7):

-Prepare 800 ml of distilled water in a suitable beaker

-Add 9.34g of Potassium phosphate dibasic (0.05364M, mw: 174.18g/mol) to the solution

-Add 6.31g of Potassium phosphate monobasic (0.0436M, mw: 136.09 g/mol) to the solution

-Add water until the volume reach 1L

-Put it in Autoclave (1h) for microbial usage.

1.1.3 Preparation of the turbidity calibration 0.5 McFarland:(40)

1. we added 0.5 mL of a 0.048 mol/L solution of BaCl₂ (1.175% w/v BaCl₂ 2H₂O) to 99.5 mL of a 0.18 mol/L solution (0.36 N) of H₂SO₄ (1% v/v) and we shook vigorously
2. We checked the density of the suspension using a spectrophotometer with a 1 cm beam and matching cuvettes. The absorbance at 625 nm should be between 0.08 and 0.13

3. We distributed the suspension in tubes of the same size as those used to adjust the inoculum and then we sealed the tubes
4. Once sealed, we stored these tubes at room temperature and protected from light. Before use, we mixed the tube vigorously using a Vortex (6 months' storage)



1.1.4 Prices of laboratory products:

	Quantity	Company supplier	Price
Tryptone	250g	Merc	86.3\$
Glucose	1Kg	Merc	56.5\$
Lactose	500g	Ali express	72.5\$
Immobilized penicillin G acylase	10g	Biosynth	69.3\$

1.1.5 MEGBI-Suppliers contact:

Contact List		
Chemical reagent	Laboratory equipment	Drugs (Ministry of Health website)
-Biosynth.com (Shipping from Germany)	-Medi lab c.rached@medilab.com	https://www.moph.gov.lb/ar/Drugs/index/0/7963
-ibra@ibrahadad.com patriciazaczac@ibrahadad.com 01-901324 01-901325 Patricia: 03-971430	-Burhan Kabbara 03-339523	
-Vtc lab Mark@vtc-lb.com	-Firas Zakariya (CityMed) 81-879064	

