بسم الله الرحمن الرحيم

AECENAR
Association for Economical and Technological Cooperation in the Euro-Asian and North-African Region
TECDA

MEGBI - Middle East Genetics and Biotechnology Institute
مركز أبحاث للجينات والتقنية البيولوجية
http://aecenar.com/institutes/megbi

MEGBI Final Report (2021 – 2024)

Last Update: 08.04.2025 18:23

Editor: Dr. Samir Mourad

GREEN CHEMISTRY

MEGBI Actual Status Apr 2025

Legend:

- Still open
- In work
- completed

Still open issues:

For Aspirin Needed Chemicals + Aspirin Production Plant
- Responsible: Ihab W./GreenChemistry
- Period: Apr-Sep 2025
- Needed Budget: 6400$
- *Location: Ras Nhache, 3mx12m(6mx6m)*

Penicillin/Ampicillin Production
- Responsible: Green Chemistry
- Period: Oct - Dec 2025
- Needed Budget: 3,200$

Monoclonal Antibodies Production
- Responsible: Green Chemistry
- Due Date/Timeline: 2026
- Needed Budget: 35,000$

Genetic Engineering Lab (Ras Nhache)

Responsible: Muh Qalawoun
Rent 6/24-12/24: 6x150$ =900$
Rent 2025: 1800$

Ras Nhache Apr-Sep 25

Aspirin Production Pilot Plant

Due Date: 9/24
Responsible: Ihab W. GreenChemistry
Still needed Budget: 1000$

Department	Open Cost	Remarks	Due Date	Status
System Design				Done
Realization	1000$	Change steel parts to stainless steel		In work
AUT				Done
Systemtest				open

For aspirin needed chemicals Production Plant (Green Chemistry)

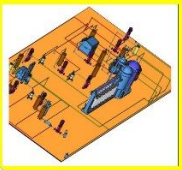
Due Date: 9/25
Responsible: Ihab W./GreenChemistry
Still needed Budget: 200$x2x6(Staff) + 4x1000$(Mat.) = 6400$

Department	Open Cost	Remarks	Status
System Design			In work
Realization	1500$		In work
AUT Acetic Anh., Acetyl Acid	1000$		Done
Systemtest	1000$		open

Penicillin/Ampicillin Production Pilot Plant Ras Nhache Oct-Dec 25

Department	Open Cost	Remarks	Status
System Design			In work
Realization	30,000$		In work
AUT			Done
Systemtest	1000$		open

HBV Vaccine Pilot Plant (Due Date: 2026)



Task	Responsible	Status
Systemdesign		In work
Realization		open
AUT		GUI done
Commisioning		

Monoclonal Antibodies Production Site

The following reports are part of the final report:

MEGBI-APP (Antibiotitics Pilot Plant) Report 2021

Ampicillin Production With Quantification of Penicillin and Ampicillin

Authors:

Amin Etro, Hind Abd El Hamid, Fatima El Hamadi, Jana Hamzeh, Ihab Wahbe, Alaa Bzal

Last Update: 01.01.2022 11:24

MEGBI-APP (Antibiotics Pilot Plant) Report 2022

Penicillin, Ampicillin, and Aspirin production and quantification

Authors:

Hind Abd El Hamid, Mohammad Kalawoun, Rayane Dergham, Jana Hamzeh, Alaa Bzal, Salwa Ossman

Last Update: 05.04.2025 19:59

MEGBI-APP (Antibiotics Pilot Plant) Report 2023

- Penicillin and Ampicillin production and quantification (Lab scale)
- Aspirin production and quantification (Lab scale and Pilot Plant Scale)
- Generic Pilot Plant Design for Penicillin/Ampicillin/Aspirin Pilot Plant

Authors:

Mohammad Kalawoun, Abdullah Mourad, Ghina Abdel-Hamid, Rama Uthman, Ghinwa Younes, Cynthia Baissary, Salwa Othman

Last Update: 30.12.2023 14:14

Edited on 7.4.2025 by Dr. Samir Mourad

MEGBI REPORT 2024

Chemicals Production for Aspirin Production Plant

Editors:

Maryam El Rez, Dr. Samir Mourad

With the contributions of:

Muhamad Kalaoun, Abdullah Mourad, Nour Koulayb, Ahmad Jawhar, Ali Foul, Rawan Abdelmajid

Last Update: 24.03.2025 15:17

With contributions of: Hind Abd El Hamid, Mohammad Kalawoun, Abdullah Mourad, Amin Etro, Ihab Wahbe, Fatima El Hamadi, Jana Ham zeh, Alaa Bzal, Rayane Dergham, Salwa Ossman, Ghina Abdel-Hamid, Rama Uthman, Ghinwa Younes, Cynthia Baissary, Salwa Othman, Nour Koulayb, Ahmad Jawhar, Ali Foul, Rawan Abdelmajid

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Ras Nhache Apr-Sep 25

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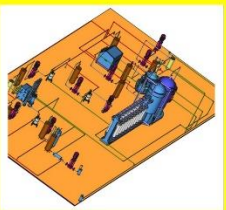

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Systemdesign		In work
Realization		open
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Commisioning		

Monoclonal Antibodies Production Site

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Content in Short

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MEGBI-APP (Antibiotitics Pilot Plant) Report 2021

Ampicillin Production With Quantification of Penicillin and Ampicillin

Authors:

Amin Etro
Hind Abd El Hamid
Fatima El Hamadi
Jana Hamzeh
Ihab Wahbe
Alaa Bzal

Last Update: 01.01.2022 11:24

1 Introduction

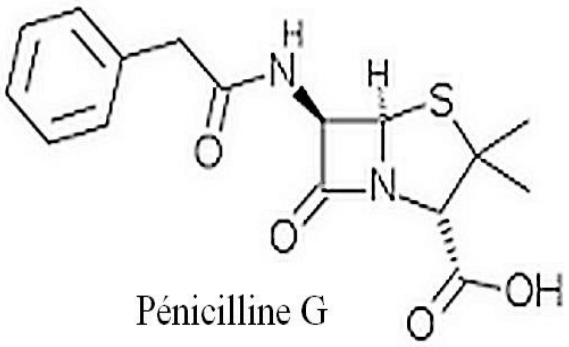
Antibiotics, in one form or another, have been in use for centuries. The vast majority of novel antibiotics have been detected by screening of "wild isolates" obtained from soil and other natural habitats. With advances in organic chemistry many antibiotics are now also obtained by chemical synthesis. Drugs used in the chemotherapy of infectious diseases are classified into two groups. Drugs that have been synthesized by chemical procedures in the laboratory are called synthetic drugs while those produced by bacteria and fungi are called antibiotics. The antibiotics are widely distributed in the nature, where they play an important role in regulating the microbial population of soil, water, sewage, and compost. Of the several hundred naturally produced antibiotics that have been purified, only a few have been sufficiently non-toxic to be of use in medical practice. Penicillin was discovered accidentally in 1928 by Fleming, who showed its efficacy in laboratory cultures against many disease producing bacteria. This discovery marked the beginning of the development of antibacterial compounds produced by living organisms(1). Microbes develop resistance through various mechanisms such as altering the target, hydrolysis, efflux, glycosylation, phosphorylation, reprogramming peptidoglycan biosynthesis, ADP-ribosylation, nucleotidylation, monooxygenation and acetylation. Resistant infections are turning deadly(2).

Semi-synthetic penicillins antibiotics (SSPAs), one of the mostimportant families of anti-infection drugs in the world market, are mainly producedby a two-step fashion(3). Ampicillin is one of the most widely used -lactam antibiotics in therapy as it is suitable for a wide spectrum of bacterial infections and has a good level of activity and tolerability(4)

1.1 Penicillin

1.1.1 What is Penicillin?

Penicillins (P, PCN or PEN) are a group of antibiotics originally derived from Penicillium moulds (principally, P. chrysogenum, P. notatum and P. rubens). The discovery and manufacture of penicillins have changed the face of medicine. The several kinds of penicillin synthesized by various species of the mold *Penicillium* may be divided into two classes: the naturally occurring penicillins (those formed during the process of mold fermentation) and the semisynthetic penicillins (those in which the structure of a chemical substance—6-aminopenicillanic acid—found in all penicillins is altered in various ways). It is the first medications to be effective against many bacterial infections caused by staphylococci and streptococci , it still widely used today though many types of bacteria have developed resistance following extensive use. Because it is possible to change the characteristics of the antibiotic, different types of penicillin are produced for different therapeutic purposes(5).



Pénicilline G

Figure 1: Penicillin G structure

1.1.2 Characterization of purified penicillin:

Characterization of purified extract and crude extract of penicillin was finally analyzed for its activity on three different pathogenic organisms, *i.e*., *Klebsella* spp., wild strain of *Escherichia coli* (*E. coli*), and methycillin resistant *Staphylococcus aureus* (MRSA). A bacterial lawn of the foresaid bacterial species was spread on nutrient agar plate and a well was bored on the bacterial agar plates. And randomly selected samples which had a high rate of inhibition during routine assay were used for characterization. 100 µL of the samples from crude and purified extract were loaded in two different wells bored in a single plate. The plates were kept for inhibition at 37 °C for 16 to 24 h, and the results were noted(6).

1.1.3 Penicillin production qualitative analysis:

1.1.3.1 Microbiological assay

The qualitative analysis was done through β-lactamase test using penicillin resistant *Staphylococcus aureus*. Briefly, filter paper was soaked in 0.2% bromophenol blue and 2% culture sample from different shake flask culture medium. The filter paper was dried and loopful culture of penicillin resistant *S. aureus* was placed on it. The change in color was noted to see the presence of β-lactamase enzyme and to confirm the penicillin production. Quantitative analysis was performed by measuring the diameter of zones of inhibition of all the culture samples and comparing them with the standard curve drawn by measuring the diameter of zones of inhibition of standard dilutions of commercially available penicillin G(7).

Antibiotic diffusion assays are based on the technique of allowing an antibiotic to diffuse through an agar gel which has been previously seeded with a sensitive test organism. This diffusion may be of two types: (a) linear diffusion, by bringing the antibiotic in contact with a column of seeded agar in a capillary or test tube; and (b) radial diffusion around a suitable reservoir on a seeded agar plate. Linear diffusion methods have been developed by both Japanese and American workers for penicillin and streptomycin; however, linear diffusion techniques require specialized equipment and are not in general use. The plate assay method for antibiotics is the most widely used and accepted method employing the diffusion technique. Its advantages lie in its simplicity as to labor and equipment. It has definite disadvantages in that the assay is affected by various salts, surface active agents, and solvents which tend to change diffusion characteristics of the antibiotics. With

alterations in the diffusion characteristics the dose response curves of the sample and standard will no longer be parallel and the assay itself would be invalid. The distribution of an antibiotic in the agar around a reservoir can be expressed theoretically by an equation involving the initial quantity of antibiotic, the depth of the agar layer, the diffusion constant, the concentration at a given distance from the container, and the time of diffusion. Theory predicts that the square of the diameter of the inhibition zone will be proportional to the logarithm of the antibiotic concentration. This relationship has been found to hold for most antibiotics. Good assay plate methods are available for penicillin, streptomycin, bacitracin, and polymyxin; however, the newer broad spectrum antibiotics tend to give poorly defined zone edges on assay plates(8).

1.1.3.2 Factors influencing variability and error in microbiological assays

Microbiological assay provides a valid measure of antibiotic activity with some problem of interference from biologically active compounds or degraded products. Several factors are investigated by scientists that normally cause variation in zone diameters in conventional agar diffusion bioassay. Among these factors the most considerable is the unequal exposure of the individual plates at the top or bottom of the stacks. Another major variable is the variable in the time interval between pouring seeded agar in the plates and the time of applying the solution of the antibiotic to the plates. Other factors that lead to variability and error in microbiological assay include agar thickness, inoculums concentration, incubation temperature, exposure-time duration and sample preparation. Factors affecting microbial growth rates include pH and chemical composition of media and pH of buffer solution used(9).

HPLC is a chromatographic technique used for the identification, quantification and purification of individual components of a mixture in analytical chemistry. HPLC is used extensively throughout the pharmaceutical industries for the quantification of antibiotics in pharmaceutical preparations. It is used to provide information on the composition of drug related samples.

The information obtained may be qualitative, indicating what compounds are present in the sample, or quantitative, providing the actual amounts of compounds in the sample. HPLC is used at all the different stages in the creation of a new drug, and is also used routinely during drug manufacturing. It is more attractive than the classical bioassay in terms of speed, accuracy and precision. Hence, it has largely replaced the microbiological assays to determine the antibiotic concentrations in body fluids and pharmaceutical preparations(9). HPLC analysis of penicillin was carried out with UV detector set at 254 nm. The column used for analysis is C-18. The mobile phase consisted of methanol: phosphate buffer (85:15, v/v) at flow rate 1 mL/min. Standard used for comparison is Pencom®13 (commercially available penicillin injection)(6).

The literature finding shows that both microbiological assay and HPLC method exhibit several advantages and inadequacies. Although HPLC method is fast, accurate and precise for quantification of potency of antibiotics, it cannot determine bioactivity. However, microbiological assay is simple, sensitive, accurate, precise and cost effective to estimate both potency and bioactivity. Besides this, microbiological assay become the most important method to quantify the

concentration of active ingredient required for the inhibition of growth of antibiotic resistant microorganism(9).

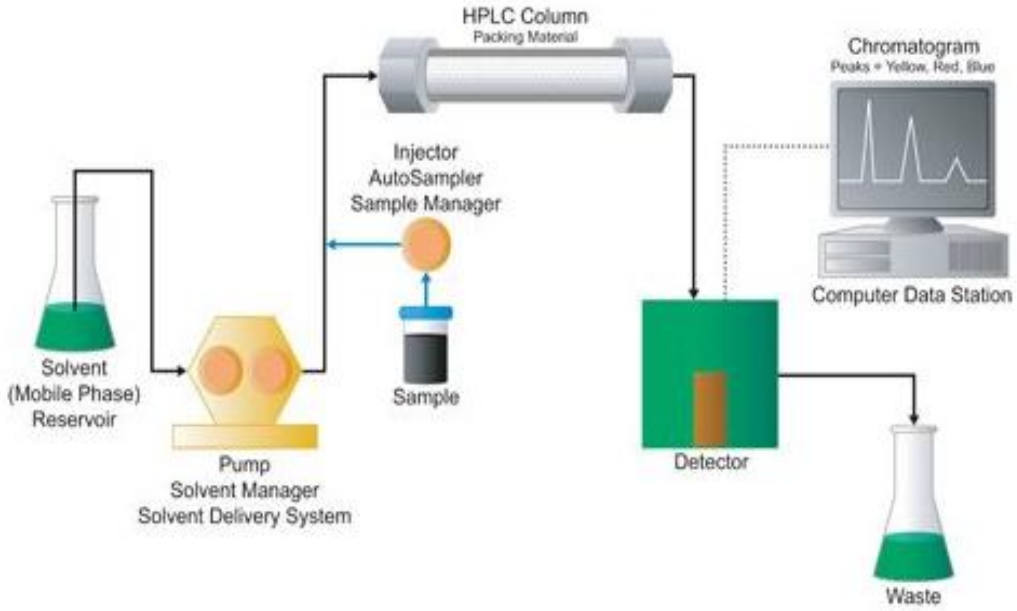


Figure 2: HPLC system

1.2 Ampicillin

1.2.1 What is Ampicillin?

Ampicillin is an antibiotic used to prevent and treat a number of bacterial infections, such as respiratory tract infections, urinary tract infections, meningitis, salmonellosis, and endocarditis. It may also be used to prevent group B streptococcal infection in newborns.[3]

It is used by mouth, by injection into a muscle, or intravenously. Common side effects include rash, nausea, and diarrhea. It should not be used in people who are allergic to penicillin. Serious side effects may include Clostridium difficile colitis or anaphylaxis.[3] While usable in those with kidney problems, the dose may need to be decreased. Its use during pregnancy and breast-feeding appears to be generally safe. Ampicillin was discovered in 1958 and came into commercial use in 1961. It is on the World Health Organization's List of Essential Medicines. The World Health Organization classifies ampicillin as critically important for human medicine. It is available as a generic medication(10).

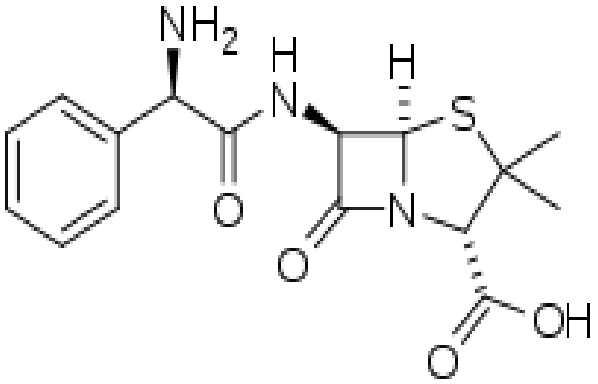


Figure 3: Ampicillin structure

1.2.2 harmacology

Mechanism of Action:

Ampicillin is in the penicillin group of beta-lactam antibiotics and is part of the amino-penicillin family. It is roughly equivalent to amoxicillin in terms of activity. Ampicillin is able to penetrate Gram-positive and some Gram-negative bacteria. It differs from penicillin G, or benzylpenicillin, only by the presence of an amino group. This amino group, present on both ampicillin and amoxicillin, helps these antibiotics pass through the pores of the outer membrane of Gram-negative bacteria, such as *E.coli*, *Proteus mirabilis*, *Salmonella enterica*, and *Shigella*.

Ampicillin acts as an irreversible inhibitor of the enzyme trans-peptidase, which is needed by bacteria to make the cell wall. It inhibits the third and final stage of bacterial cell wall synthesis in binary fission, which ultimately leads to cell lysis; therefore, ampicillin is usually bacteriolytic(10).

1.2.3 Pharmacokinetics

Ampicillin is well-absorbed from the GI tract (though food reduces its absorption), and reaches peak concentrations in one to two hours. The bioavailability is around 62% for parenteral routes. Unlike other penicillins, which usually bind 60–90% to plasma proteins, ampicillin binds to only 15–20%.

Ampicillin is distributed through most tissues, though it is concentrated in the liver and kidneys. It can also be found in the cerebrospinal fluid when the meninges become inflamed (such as, for example, meningitis). Some ampicillin is metabolized by hydrolyzing the beta-lactam ring to penicilloic acid, though most of it is excreted unchanged. In the kidneys, it is filtered out mostly by tubular secretion; some also undergoes glomerular filtration, and the rest is excreted in the feces and bile(10).

1.2.4 Side effects

Ampicillin is comparatively less toxic than other antibiotics, and side effects are more likely in those who are sensitive to penicillin and those with a history of asthma or allergies. In very rare cases, it causes severe side effects such as angioedema, anaphylaxis, and *C. difficile* infection (that can range from mild diarrhea to serious pseudomembranous colitis). Some develop black "furry" tongue. Serious adverse effects also include seizures and serum sickness. The most common side effects, experienced by about 10% of users are diarrhea and rash. Less common side effects can

be nausea, vomiting, itching, and blood dyscrasias. The gastrointestinal effects, such as hairy tongue, nausea, vomiting, diarrhea, and colitis, are more common with the oral form of penicillin. Other conditions may develop up several weeks after treatment(10).

1.2.5 Overdose

Ampicillin overdose can cause behavioral changes, confusion, blackouts, and convulsions, as well as neuromuscular hypersensitivity, electrolyte imbalance, and kidney failure(10).

2 Devices, Materials and Methods

2.1 Penicillin Production

In the first, we put an orange and a half of bread in a fermentation conditions until will be able to see many fermented regions.

All used lab glassware are sterilized by adding some ml of water, covering with metallic paper and bowling until the water are totally evaporates

Microbial essays (culture and inoculation) are performed in a sterile area near a flame

2.1.1 Preparation of agarose gel

- We try to melt two tryptone tubes by using a heated water (bain marie)
- We weigh 1 g of glucose powder
- In an erlenmeyer flask we mix the melted tryptone, the glucose and 20 ml of distilled water
- We keep heating until we get a homogeneous mixture
- We fill the mixture in two petri dishes
- We heat them in a pressure cooker after boiling for 15 min
- We let them cool down and we wait about 30 min until the gel are totally solidified
- We put them in the fridge until the time of microbial cultivation

2.1.2 Microbial culture

- We cultivate the two petri dishes differently with the two used strains of penicillium

We incubatated them at room temperature for 7 days

2.1.3 Preparation of liquid medium

- We weigh 4g of glucose powder, 4 g of lactose, 2g of peptone, 0.2g of $MgCl_2$, 0.2g of KCl, and 1g of KH_2PO_4
- We add 200ml of distilled water
- We distribute the mixture in 2 erlenmeyer flasks (100 ml) with an equal proportion
- We heat them with mixing for 15min by using a magnetic hot plate stirrer
- We let them cool down for about 30 min
- We inoculate each of them with one of the two cultivated petri dishes already prepared (about two to three colony for each of them)
- We incubate them at room temperature for 7days (bread penicillium with shaking and the other lemon penicillium without shaking)

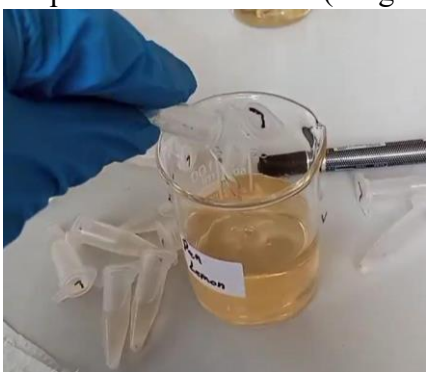
2.1.4 Filtration and the adding of ethyl acetate

- After 7 days of incubation in liquid medium we filter the two inoculated liquid medium by using of filter paper
- Then in each of the obtaining filtrate we add 0.43g of charcoal and 0.5g of KH_2PO_4 and we leave them for 20 min
- We decant the liquid from the charcoal, then we add ethyl acetate(proportion 50/50)
- (we have used 60ml of lemon penicillim filtrate with 60 ml of ethyl acetate and 46 ml of bread penicillium filtrate with 46 ml of ethyl acetate)
- We incubate them in the fridge some days (about 7 days).

2.1.5 Purification

1. Here the penicillin was dissolved in ethyl acetate
2. The centrifugation method was applied to eliminate the pellet containing cells debris and all other contaminant (4000x for 15 min)

3. The supernatant is reserved (we got about 60 ml of supernatant for each species of penicillium)



4. We add 5g of sodium bicarbonate for each supernatant to obtain the penicillium in salt form.

5. We left it in the fridge for a few days to precipitate the penicillium salt

2.2 Penicilin Quantification Proposed experimental protocol:

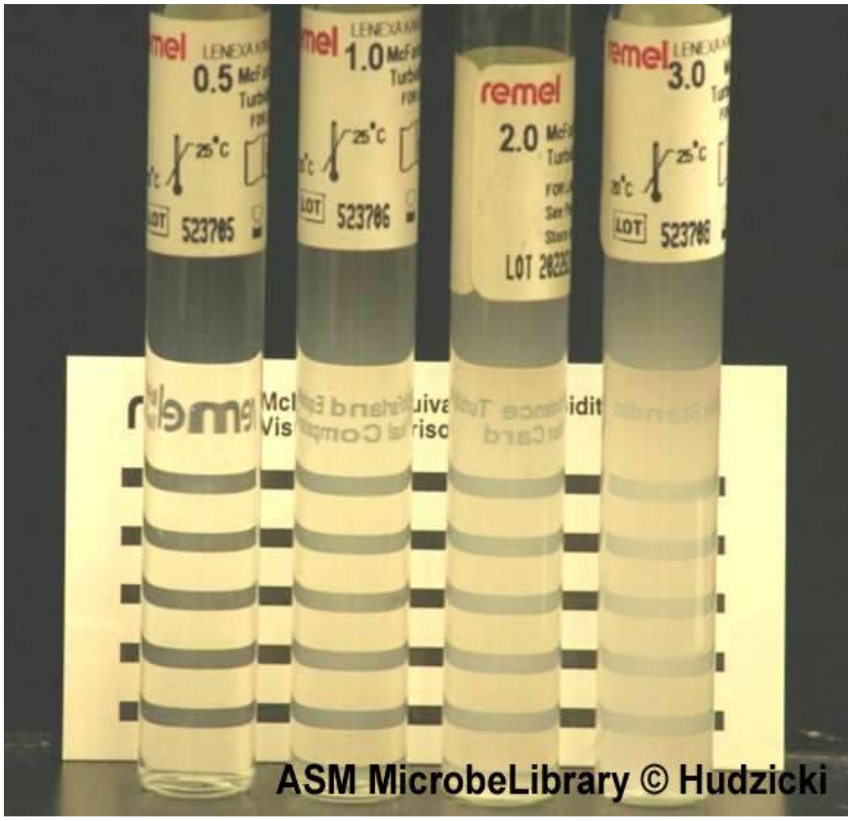
2.2.1 Preparation of the turbidity calibration 0.5 McFarland(11):

1. we added 0.5 mL of a 0.048 mol/L solution of $BaCl_2$ (1.175% w/v $BaCl_2$ $2H_2O$) to 99.5 mL of a 0.18 mol/L solution (0.36 N) of H_2SO_4 (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)



2.2.2 Quantification of the produced penicillin using the disk diffusion method (Kirby-Bauer Test)(12):

2.2.2.1 Preparation of the inoculum:

1) We took 3 to 5 colonies of the isolated colonies with a loop, and we added them in 2ml sterile saline (NaCl 0.9%)

2) We Vortex the saline tube to create a smooth suspension.

3) We 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.

4) Use this suspension within 15 minutes of preparation.

5) We inoculate the surface of 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

6) We allow the plate to sit 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

2.2.2.2 Preparation of the disks:

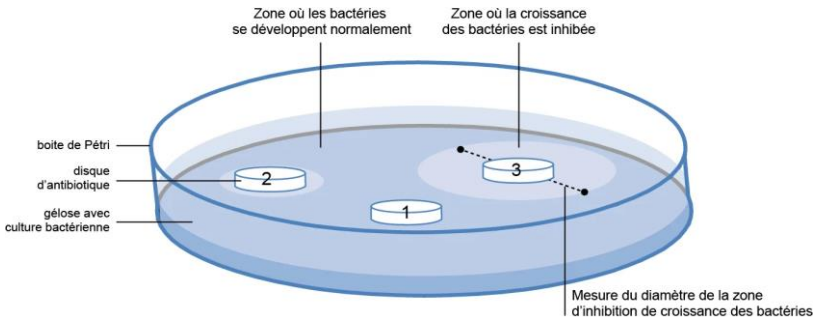
1) We dilute the standard penicillin 10 times (Concentrations: 1.5; 1.4; 1.3; 1.2; 1.1; 1.0; 0.9; 0.8; 0.7; 0.6) to obtain different concentrations

2) We dip each of the 10 discs in one of the 10 concentrations of penicillin

3) We dip another one disk in the unknown produced penicillin

4) We distribute the 11 disks in plates at a distance of (26) mm apart

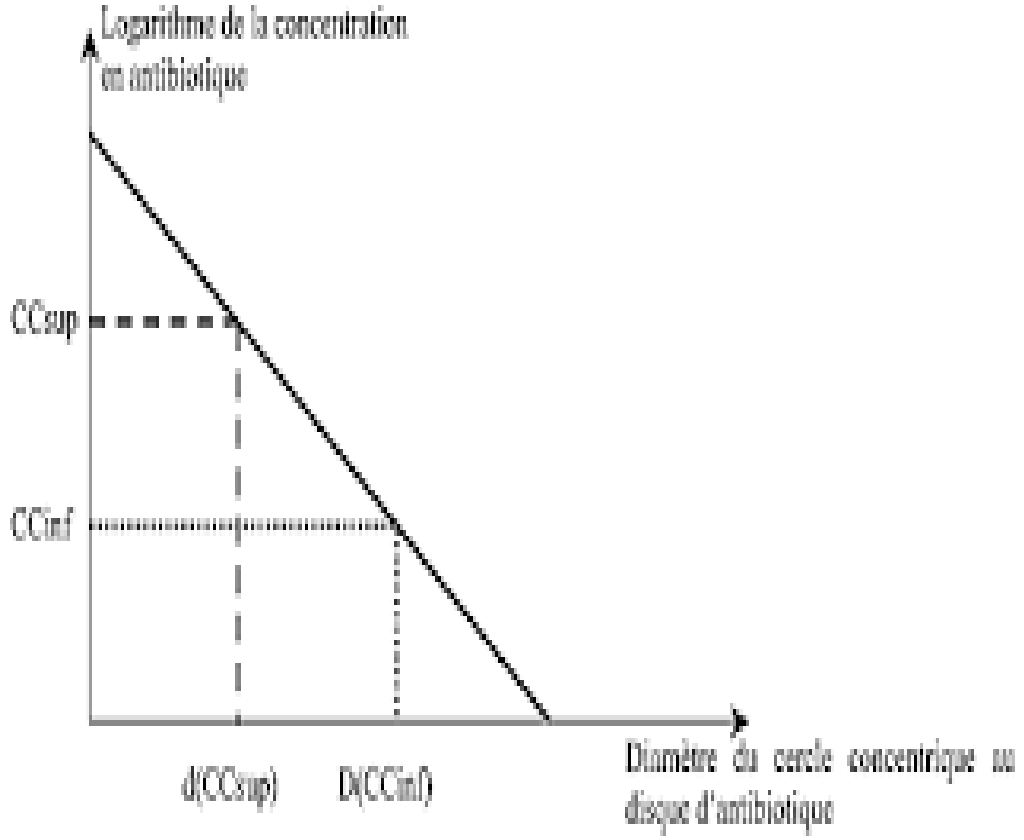
5) Once all disks are in place, we replaced the lid, inverted the plate and placed it at 37°C for 18 to 24 hours



2.2.2.3 Quantification of the produced penicillin:

1) After the growth time, we measured the zone of inhibition that had appeared using a ruler

2) We drew a graph showing the concentration of penicillin as a function of the diameter in order to be able to quantify the produced penicillin (Log C as a function of diameter)



2.3 Ampicillin production proposed protocol

To produce semi-synthetic β-lactam(Ampicillin), there are two proposed methods: One put-one step synthesis(1P1S) and one put two step synthesis while the second has showed a most overall yield then the first:

2.3.1 1P1S:

Pen G: 15 ml of 20 mM

D-PGME: 60 mM in 100 mM Phosphate buffer;PH7

iPGA(99.2 UPenG)

We add all the materials in round bottom flask on magnetic stir plate at T°(22-25°C) about 160min

2.3.2 1P2S:

Pen G: 7.5ml of 40mM

Phosphate buffer: 100mM; PH7

iPGA:124UPenG/gram of carrier

We add all the materials in round bottom flask on magnetic stir plate at T°(22-25°C). Then after about 60 min we add D-PGME(7.5 ml/120 mM).

Then after 160 min the PH was adjusted with NaOH from approximately 6.4-7.0

The two-enzyme system with iPGA and AEH outperformed the systems that used only iPGA, thus demonstrating the clear advantage of using AEH(1). This result can be shown at the figure below :Figure 1

AEH: Soluble amino ester hydrolase from *Xanthomonas campestris pv. Campestris*

iPGA: Eupergit-immobilized penicillin G acylase from *Escherichia coli*

D-PGME: (D)-phenylglycine methyl ester hydrochloride

2.4 Proposed protocol for ampicillin quantification

2.4.1 Preparation of the turbidity calibration 0.5 McFarland(11):

1. we add 0.5 mL of a 0.048 mol/L solution of $BaCl_2$ (1.175% w/v $BaCl_2$ $2H_2O$) to 99.5 mL of a 0.18 mol/L solution (0.36 N) of H_2SO_4 (1% v/v) and we shook vigorously

2. We check 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 distribute the suspension in tubes of the same size as those used to adjust the inoculum and then we seal the tubes

4. Once sealed, we store these tubes at room temperature and protect from light. Before use, we mix the tube vigorously using a Vortex (6 months' storage)

2.4.2 The three bacterial strains which can be used:

E.*coli* / S.*aureus* / S.*pneumonia*

2.4.3 Quantification of the produced ampicillin using the disk diffusion method:

2.4.3.1 Preparation of the inoculum:

1) We took 3 to 5 colonies of the isolated colonies with a loop, and we added them in 2ml sterile saline (NaCl 0.9%)

2) We Vortex the saline tube to create a smooth suspension.

3) We 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.

4) Use this suspension within 15 minutes of preparation.

5) We inoculate the surface of 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

6) We allow the plate to sit 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

2.4.3.2 Preparation of the disks:

1) We dilute the standard ampicillin 10 times (Concentrations: 1.5; 1.4; 1.3; 1.2; 1.1; 1.0; 0.9; 0.8; 0.7; 0.6) to obtain different concentrations

2) We dip each of the 10 discs in one of the 10 concentrations of ampicillin

3) We dip another one disk in the unknown produced ampicillin

4) We distribute the 11 disks in plates at a distance of (26) mm apart

5) Once all disks are in place, we replaced the lid, inverted the plate and placed it at 37^{o}C for 18 to 24 hours

2.4.3.3 Quantification of the produced ampicillin:

1) After the growth time, we measured the zone of inhibition that had appeared using a ruler

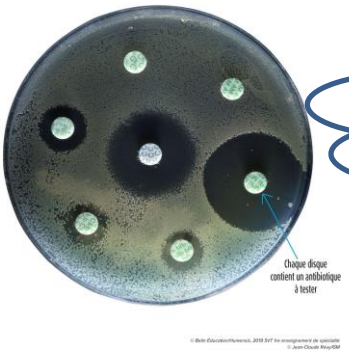
2) We drew a graph showing the concentration of ampicillin as a function of the diameter in order to be able to quantify the produced ampicillin (Log C as a function of diameter)

2.5 Penicillin Production (Plant Scale)

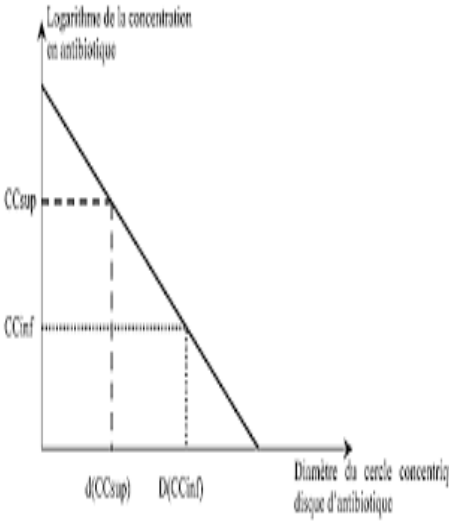
2.5.1 Quantification of produced penicillin G:

3 principle methods to quantify the penicillin

- High performance liquid
- Liquid chromatography with tandem mass spectroscopy (LC-MS/MS)
- Determination of penicillin concentration by biological assay

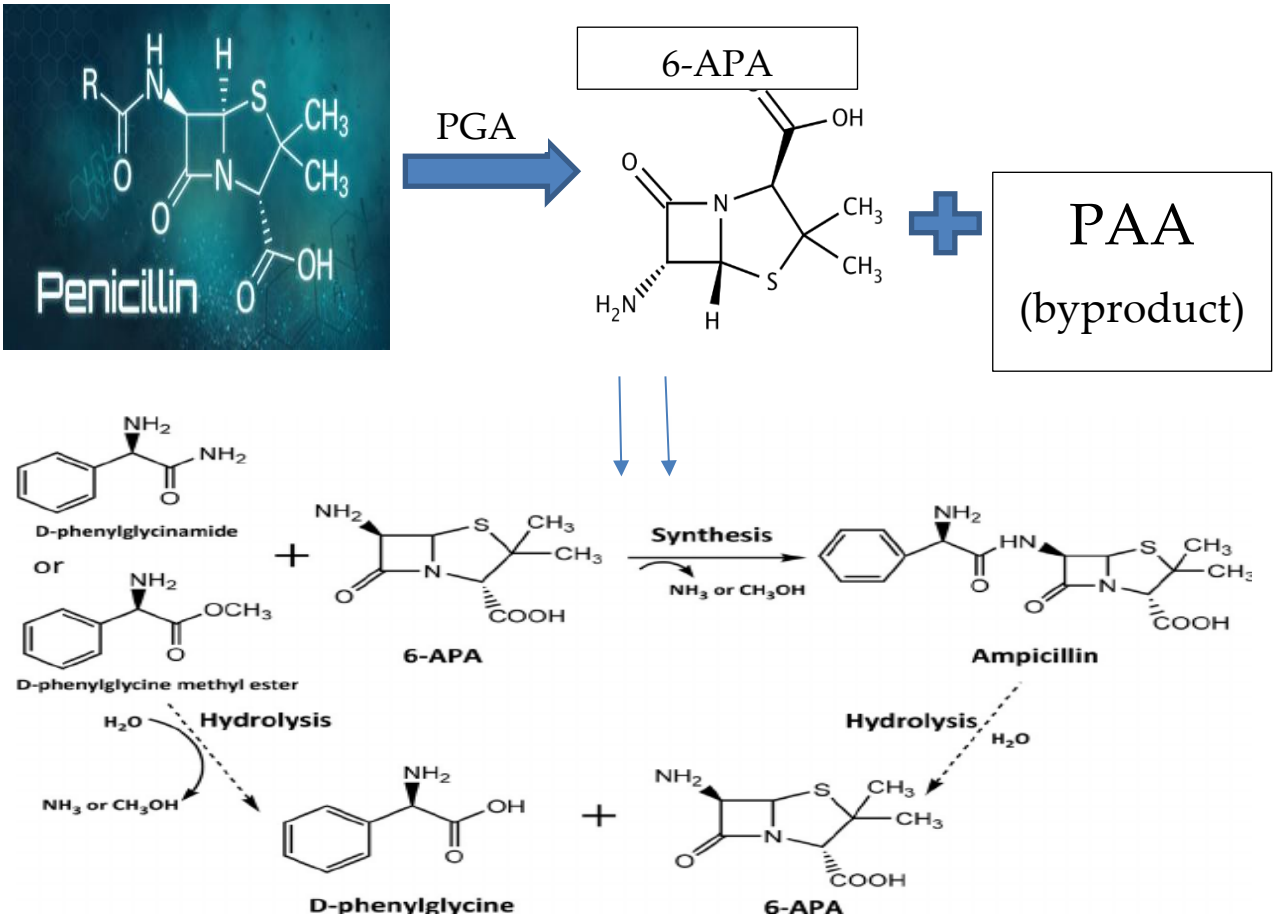


We can determine the concentration of produced penicillin by the reading of inhibition zones of different known concentration of standard



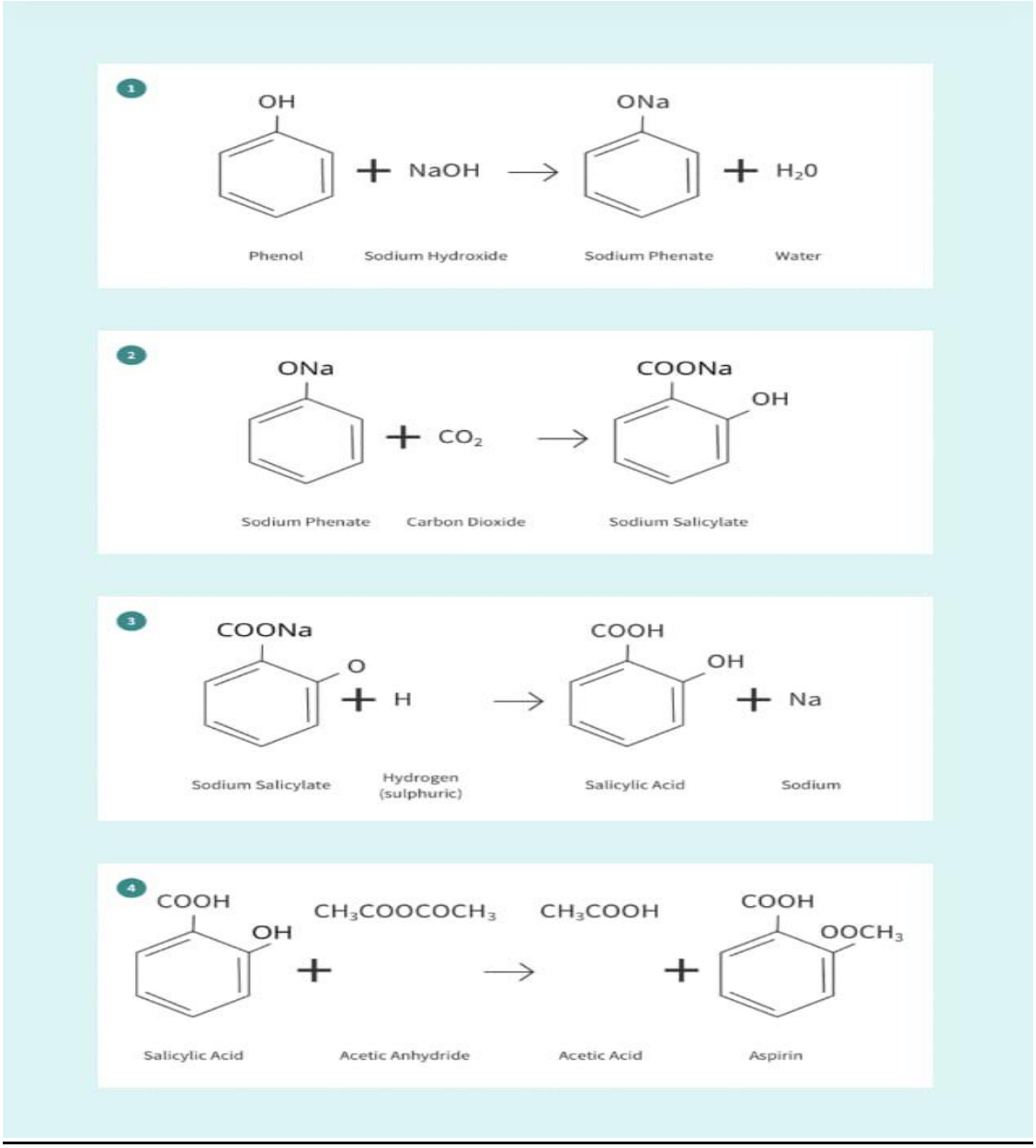
- We need a sensitive microbial organism such as: klebsiella, E.coli or MRSA
- We need different penicillin-ATB disk with different concentration
- Then we can determine the unknown concentration of the produced penicillin by comparing with the obtained standard curve

2.5.2 Production of ampicillin from penicillin



2.5.3 Ampicillin quantification

Similar to penicillin quantification

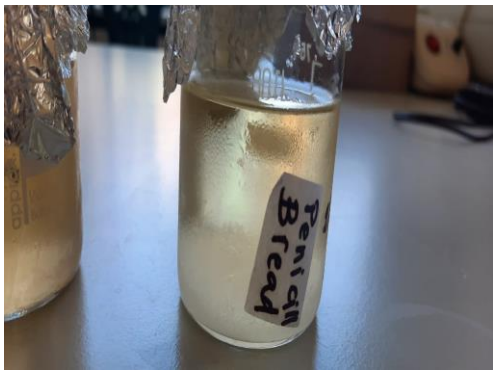


1- Production of bioethanol from molasses using a special species of yeast

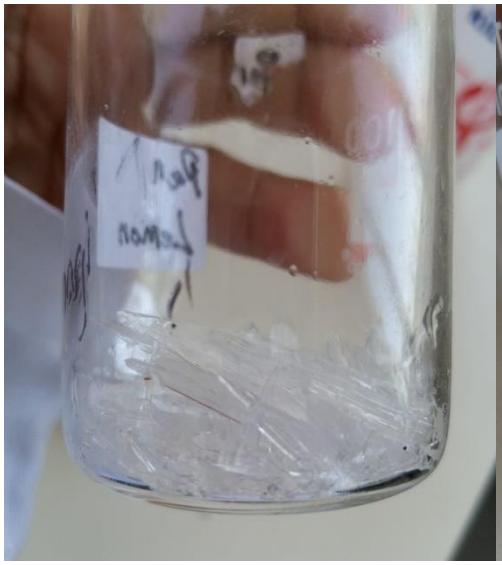
3 Results

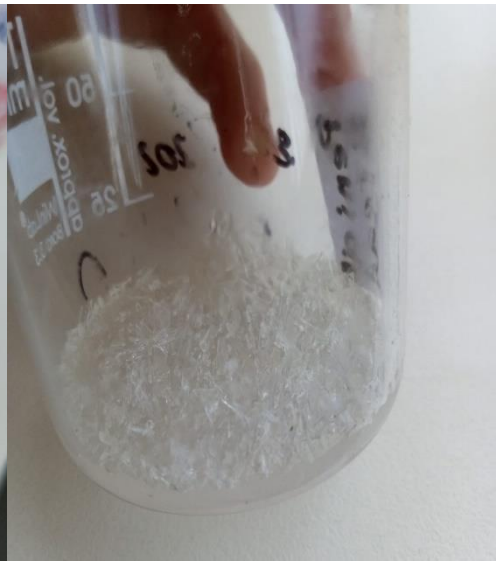
3.1 Penicillin Production

After cooling about 7 days we obtained penicillin crystals.



We poured the liquid throughly to remove it, then we kept the crystals in the fridge in order to dry them well.





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5 Master thesis tasks

1- **Quantification of produced penicillin G**:

Our laboratory had developed a machine to produce penicillin from P.*Chrysogenum.* After we succeeded in its manufacturing process, we try to quantify our product. We find there is three recurrent way to quantify penicillin: HPLC ; LC-MS/MS and biological assay. In this project we try to quantify the penicillin G, which was produce in last time, by using the biological assay which will enable us to determine the concentration of our produced penicillin referring on the measurement of inhibition zone resulting by different concentration of the standard penicillin G. You can find a detailed summary of the protocol below.

2- Production of ampicillin from penicillin:

Based on our produced penicillin we try to produce a semi-synthetic penicillin (Ampicillin). First we try to hydrolysis the penicillin with penicillin G acyclase (PGA), we obtain the β-lactam moiety for all penicillin: 6-aminopenicillanic acid (6-APA). Then a chemical coupling of 6-APA with an acyl side chain by using D-phenylglycine methyl ester(D-PGME) enable us to obtain finally the semi-synthetic penicillin (AMP).

3- Ampicillin quantification:

As we try to quantify the produced penicillin in the first project, here we try also to quantify the produced ampicillin and to determine the best way which can be used for this reason.

4- Aspirin production, purification and quantification:

Based on the advantage of aspirin as a pain, fever and inflammation reducing, we try to find a lab scale to produce Aspirin in our laboratory. For this chemical reaction we need: Phenol, Sodium hydroxide, Carbone dioxide, hydrogen and acetic anhydride. Then it is important to quantify our produced aspirin to know if we succeeded or not. You can find the detailed chemical reaction below

5- Production of bioethanol from molasses using a special species of yeast

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مركز أبحاث للجينات والتقنية البيولوجية

MEGBI-APP (Antibiotics/Aspirin Pilot Plant) Report 2022

Penicillin, Ampicillin, and Aspirin production and quantification

Authors:

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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 consummer to treat even to prevent several diseaes. 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 medecines like these mentionned above, for this reason the import still the ideal solution to secures the locally need. According that, we as AECENAR, and specially as MEGBI institute, we try to find and even to produce these medecines locally by developping a bioreactor which can produce this types of drugs .

6 Part I: Aspirin

6.1 Overview

6.1.1 History and usages:

In 1899, the Bayer Company in Germany patented a drug they called aspirin, which was a modification of salicylic acid. Salicylic acid contains a phenol group, and phenols are known to be irritating. The Bayer Company replaced the phenol group with an ester group. This esterified compound (acetylsalicylic acid, also known as aspirin) was shown to be much less irritating than salicylic acid(1).(Fgure1)

Aspirin or Acetylsalicylic acid (ASA) is one of the first drugs to come into common usage, still widely used around the world with approximately 40,000 tons produced globally each year(2).

Aspirin ($C_9H_8O_4$) remains on the World Health Organization's Model List of Essential Medicine (21st List 2019) both for its use in pain control and anti-platelet effects(3).

Aspirin is used to treat pain, fever, and inflammation and also to prevent heart attacks, strokes, and blood clot formation in human beingshat high risk of developing blood clots. Low doses of aspirin may be given immediately after a heart attack to reduce the risk of another heart attack or the death of heart tissue.

Aspirin may be effective at preventing certain types of cancer, particularly colorectal cancer. The aspirin belongs to a class of medications called Non-Steroidal Antiinflammatory drugs. It is transformed to the active form of salicylate in the human body. It is used to prevent swelling and phenomena related to swelling associated with trauma or allergic response. The crystals of metal complexes with aspirin have been found to be some additional medical activities such as antiulcer, anticancer, antimutagenic and antioxidative in biological systems.(4)

Our goal here is to produce aspirin with high purity in order to proceed for a pilot plant scale production and then an industrial one.

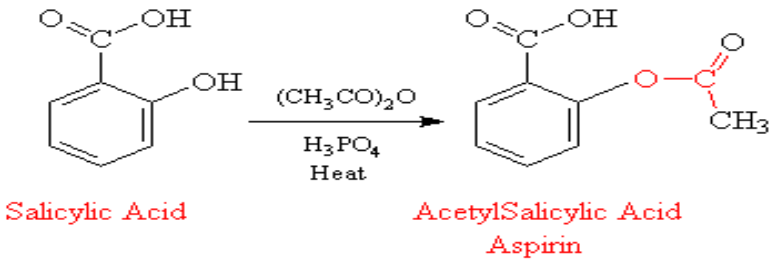


Figure 4: Salicylic acid esterification fraction

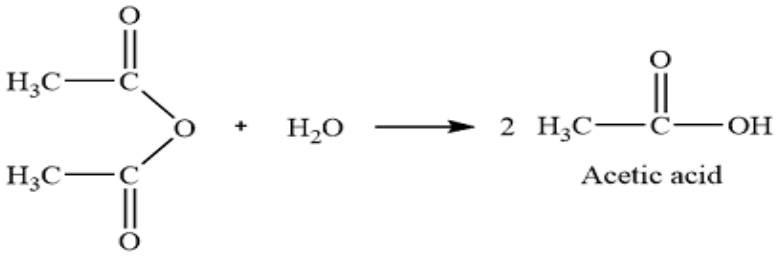
6.1.2 Safety Precautions:

• Acetic anhydride is irritating to the nose and sinuses. Keep this compound under the hood at all times, and avoid breathing the vapors.

- Sulfuric acid (H_2SO_4) is highly corrosive. Avoid contact with your eyes, skin, and clothing. In case of contact, rinse with plenty of water.
- Avoid touching the chemicals.
- Wear your safety goggles

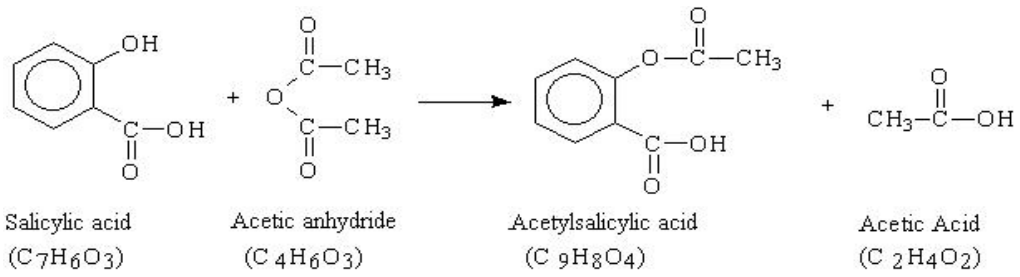
6.1.3 Overview on Aspirin steps production and Tests

In Aspirin synthesis experiment, the salicylic acid is the limiting reactant and the acetic anhydride is in excess. Aspirin, acetylsalicylic acid, can be easily made from salicylic acid by an organic reaction know as esterification. It reacts with acetic anhydride in the presence of sulfuric acid, which is a catalyst for the reaction(Figure2). After the reaction heating period is over, the excess unreacted acetic anhydride will be destroyed by the addition of water to the mixture: water reacts with acetic anhydride to form 2 molecules of acetic acid, according to the reaction shown below.(Figure3)(5)



Ace*Figure 5: Aspirin synthesis reaction*

Figure 6: Hydrolysis reaction of Acetic anhydride



6.1.3.1 Qualification test using FeCl3

The collected aspirin will be tested for its purity using FeCl3 (aq). Iron (III) ion reacts with phenols to form a purple complex. Salicylic acid contains a phenol group, but acetylsalicylic acid does not(Figure4). Therefore, if you add FeCl3 to an aspirin sample and you see a purple color, it means that there is still some salicylic acid present and the sample is impure.

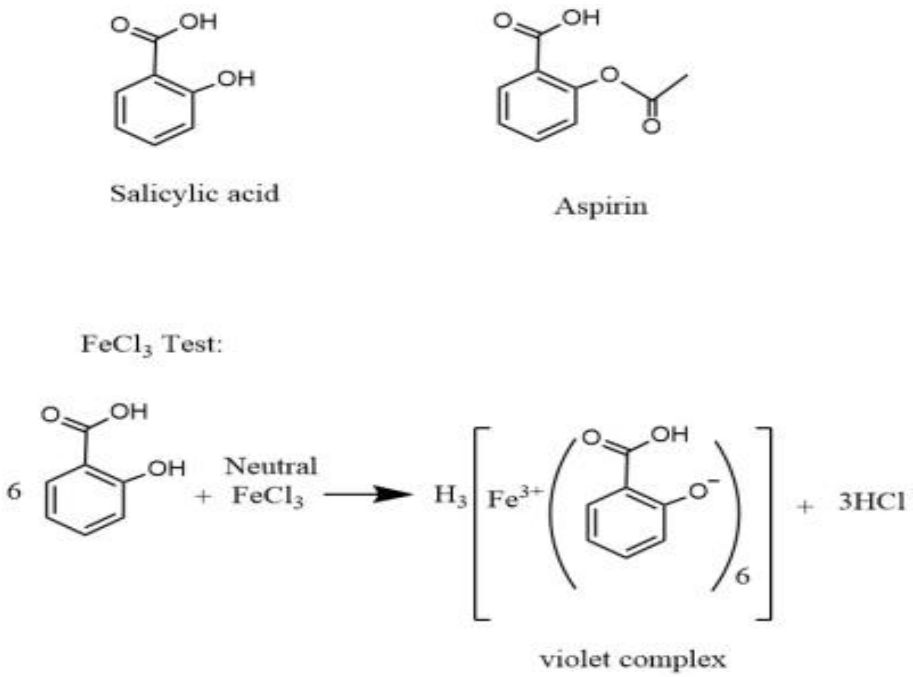


Figure 7: Salicylic acid reaction with ferric chloride

6.1.3.2 Melting point test for purity

Using a melting point apparatus, determine the melting point of your crude aspirin (In order to get a meaningful result for the melting point determination, the aspirin must be dry.)

The melting point should be recorded as a range – the first reading is the temperature at which the sample starts to liquefy, and the second reading is taken when the sample is completely melted.

The melting point of pure aspirin is 135°C, and the melting point of salicylic acid is 158°C.

Comment on the purity of produced aspirin based on its melting point.

Melting Point Analysis The melting point of the aspirin crystal is determined by using the capillary tube method. Sometimes, this study is used to differentiate the pure sample from its coformer and complex form.(4)

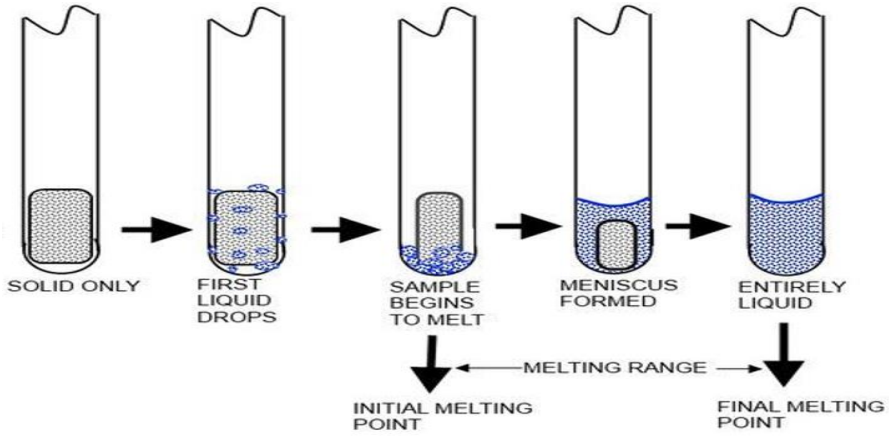


Figure 8: Melting point test

6.1.3.3 Quantitative determination of aspirin (ASA):(6)

Titrimetric method is used to determine the % purity of aspirin

Which is a basic acid base titration that envolves 3 parts , this experiment uses NaoH to titrate aspirin but before starting this titration process the NaoH must be standardized since its a secondary standard which concentration may change over time thus it must be titrated against a primary standard to ensure getting a precise concentration.

The experiment includes :

1. Standardization of NaOH solution
2. Titration of the synthesized aspirin by standardized NaoH
3. Detection of risidual salicylic acid

Procedure :

1) Standardization of NaOH solution

- Prepare the buret ; rinse it twice with distilled water and twice with NaOH then fill it with NaOH (no more than 50ml should be needed)
- Record the initial buret reading
- Weight 0.4g of KHP (Mw = 204.23g/mol) and put it in 125ml erlenmeyer flask, note the precise mass used.
- Dissolve with distilled water till 50ml and add 2 drops of phenol
- Start the titration process and note down the final buret reading when you reach end point (which is when the color remains slightly in the beaker for approximately 30 sec)
- Repeat the titration for a second run
- Calculate the molarity of NaOH solution M1 and M2
- Calculate the average molarity between the two runs M avg
- Compute the difference between the 2 runs:
  (|M1-M2| / Mavg) x 100 = % difference [if it's within 5% proceed]

2) ***Titration of aspirin by standardized NaoH***

- Weight out 0.10 – 0.15 g of aspirin into 125ml flask note down the precise mass
- Add 10 ml of 95% ethanol to dissolve the aspirin
- Add 3 – 5 drops of phenol (indicator)
- Start the titration process , record the initial and the final reading
- Report the moles of aspirin to calculate the % purity

% purity =(actual moles of aspirin / theoretical moles of aspirin) x 100

3) ***Detection of residual salicylic acid***

We use $feCl_3$ on the solution to make sure that the results are not from unreacted salicylic acid

- Weight about 0.015g of your aspirin sample
- In a test tube dissolve the aspirin with about 10 drops of ethanol
- Add 0.5ml of water
- When the solution is completely dissolved add 1-2 drops of 1% $FeCl_3$
- Record the observations
- ➢ If there is any left salicylic acid the result will be a deeply purple colored complex.

6.1.3.4 Boiling point test for aspirin purity

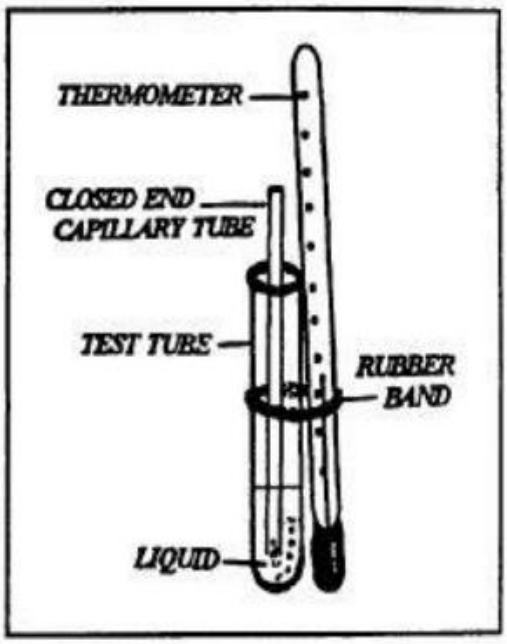
The boiling point of a compound is the temperature at which it changes from a liquid to a gas when the vapor of the liquid is equal to the atmospheric pressure. This is a physical property often used to identify substances or to check the purity of the compound. It is difficult, though, to find a boiling point. Usually, chemists can only obtain a boiling range of a 2 - 3 oC accuracy(7).

apprates / Materials:

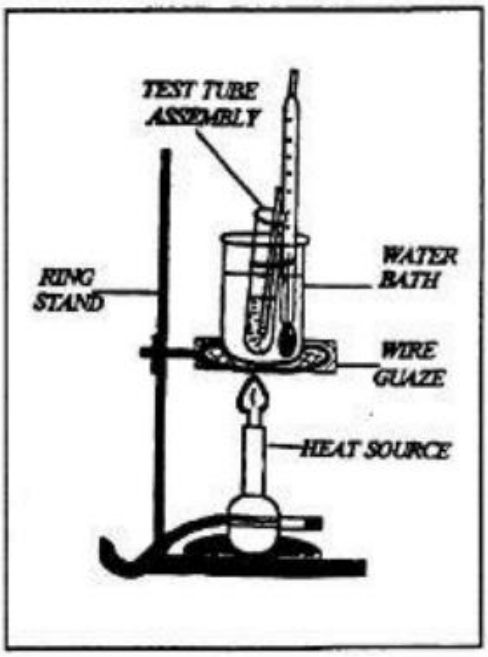
- Benzen burner
- closed end capillary tube
- thermometer
- boiling tube
- 50 mL beaker
- Tripod stand
- wire gauze
- liquid organic compounds
- glass rod
- stand and clamp
- oil bath

Procedure:

1. Place a few ml. of a known liquid organic compound in a (boiling tube).
2. Place the capillary tube into the boiling tube with the closed end upward.
3. Attach a boiling tube to the thermometer through by the ring tube.
4. Fill a 50 mL beaker 3/4 full with water, and place on the benzene burner. Carefully immerse the boiling tube and thermometer combination into the beaker of water so that the test tube is half way in the water.
5. Begin to heat the burner /water slowly. As the liquid approaches its boiling point, a few bubbles will be observed flowing out of the end of the capillary tube. When a steady stream of bubbles are observed, turn off the hot plate , When the liquid begins to flow into the capillary tube, record the temperature of the liquid as its boiling point temperature.



Test Tube Assembly ↑



Heating Assembly↑

6.2 Materials and method:

6.2.1 List of chemicals we need for this trial

- Salicylic acid
- Acetic anhydride
- Sulfuric acid H_2SO_4
- warm water
- FeCl3 Solution (2.5%)
- Ethanol 95%

6.2.2 Steps of aspirin lab scale production

We conduct a chemical reaction to produce acetylsalicylic acid ($C_9H_8O_4$)(aspirin) with two essential reagents: salicylic acid ($C_7H_6O_3$) and acetic anhydrid ($C_4H_6O_3$) in the presence of H_2SO_4 as catalyst. (8)

1. Weigh 2.027g (0.015 mole) of salicylic acid then place it in a volumetric flask.
2. In the hood, add 5 mL (0.05 mole) of acetic anhydride (the vapors of acetic anhydride are very irritating).
3. Add 5 drops of conc. H2 SO4 (use a dropper, H2 SO4 is highly corrosive), this will be the catalyst for the reaction.
4. Swirl the flask gently until the salicylic acid dissolves.
5. Heat the flask gently on the steam bath for at least 10 minutes at 85^0.
6. Allow the flask to cool at room temperature (25^o).
7. Add 10 mL of warm water and cool the mixture for about 10 min in an ice bath so aspirin can start precipitating. (Aspirin, like many other substances, is more soluble in hot water than in cold water. Therefore, to maximize the amount of crystals, it is best to cool the mixture as much as possible.)
8. Filtrate the aspirin using filter paper (add small amount of cold water on the filter paper before adding the solution).
9. Pour the crystals into a beaker. The aspirin collected will then be purified by recrystallization. In this purification method, the crude aspirin will be redissolved.
10. Dissolve them again by adding 60 ml of warm water.
11. Put the beaker in ice to recrystallize.
12. Filter the product again.

13. Carefully lift the filter paper with the crystals on it and place it on a clean watch glass.
14. Leave this aspirin under the hood to dry.
15. Weigh the aspirin on a piece of weighing paper (You will need to scrape it off of the filter paper.)

6.2.3 Tests of Aspirin

6.2.3.1 Qualification test using FeCl3

The purity of the synthesized aspirin is measured using the ferric chloride test.(9)

The test is done with the crude aspirin, pure aspirin, and the salicylic acid:

1. Take a very small amounts of the 3 products and put them in test tubes.
2. Add 1 mL of ethanol 95% to each test tube.
3. Add 1 drop of FeCl3 Solution (2.5%).
4. Shake the test tubes and see the colors.

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Aspirin Production and quantification

Introduction: Aspirin or Acetylsalicylic acid (ASA) is one of the first drugs to come into common usage, still widely used around the world with approximately 40,000 tonnes produced globally each year. Aspirin is an ingredient in many proprietary analgesic and cold/flu preparations. It is also used for the prevention of cardiovascular disease and there is growing work on its role in the prevention and management of cancer. Aspirin ($C_9H_8O_4$) remains on the World Health Organization's Model List of Essential Medicine (21st List 2019) both for its use in pain control and anti-platelet effects.

Lab scale for Aspirin Production:

Aspirin synthesis:

- Place 2.027g (0.015 mole) of salicylic acid in a volumetric flask.
- Add 5 mL (0.05 mole) of acetic anhydride, followed by 5 drops of conc. H_2SO_4 (*use a dropper, H_2SO_4 is highly corrosive*)
- Swirl the flask gently until the salicylic acid dissolves.
- Heat the flask gently on the steam bath for at least 10 minutes.
- Allow the flask to cool at room temperature.
- Add 10 mL of warm water and cool the mixture for about 10 min in an ice bath so aspirin can start precipitating.
- Filtrate the aspirin using filter paper (add small amount of cold water on the filter paper before adding the solution).
- Pour the crystals into a beaker.
- Dissolve them again by adding 60 ml of warm water.
- Put the beaker in ice to recrystallize.
- Filter the product again.

Results 1.54 g of crystal aspirin

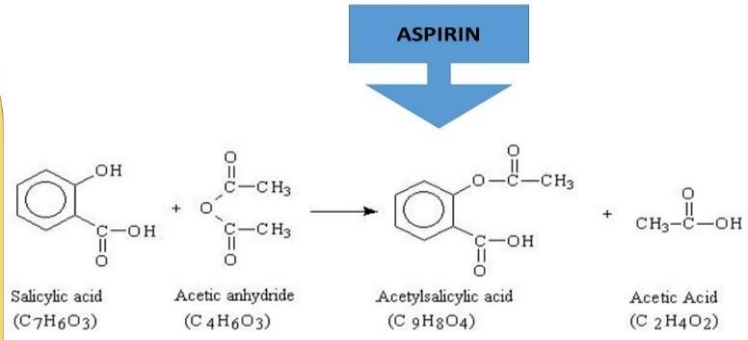
Aspirin qualitative test using ferric chloride:

The test is done with the crude product, pure product, and the salicylic acid:

- Take a very small amounts of the 3 products and put them in test tubes.
- Add 1 mL of ethanol 95% to each test tube.
- Add 1 drop of FeCl3 Solution (2.5%).
- Shake the test tubes and see the colors.
- Formation of an ironphenol complex with Fe(III) gives a definite colour ranging from red to violet, depending upon the particular phenol present.

Note: Iron (III) ion reacts with phenols to form a purple complex. Salicylic acid contains a phenol group, but acetylsalicylic acid does not.

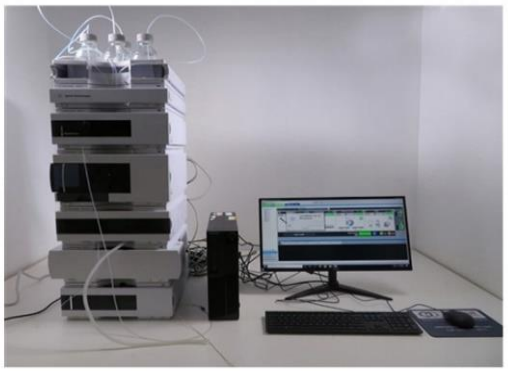
Results of qualitative test of FeCl3 with salicylic acid (purple) and aspirin (transparent)



Aspirin Quantification :

The quantification of the produced aspirin is done with HPLC wish is an analytical chemistry technique used to separate, identify, and quantify each component in the mixture.

First you run pure standard with known concentration and note down retention time and peak area.
Then run sample and note down the chromatographic area of peak appear at same retention time as that of standard.
Calculate concentration= sample Area of sample divided by area of standard multiply by conc. of standard.



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6.3 Results and discussion:

6.3.1 Yield of produced aspirin:

In the first trial of production, dry crystals of aspirin weigh 1.54g with 15min as time of crystallization without mixing.

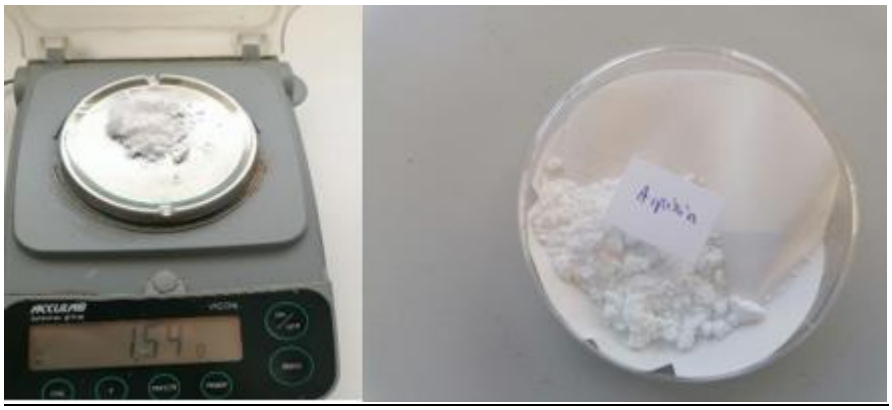

Figure 9: The crude aspirin from the first trial

In order to avoid any obstructive problems that may appear in pilot plant scale production, we repeated this trial (same procedure).

The experiment shows that there is a need to increase the time of crystallization with mixing for about 20 min so that the yield increases to 1.98g of aspirin dry crystals.

Figure 10: The crude aspirin produced in the second trial

6.3.1.1 Ferric chloride test:

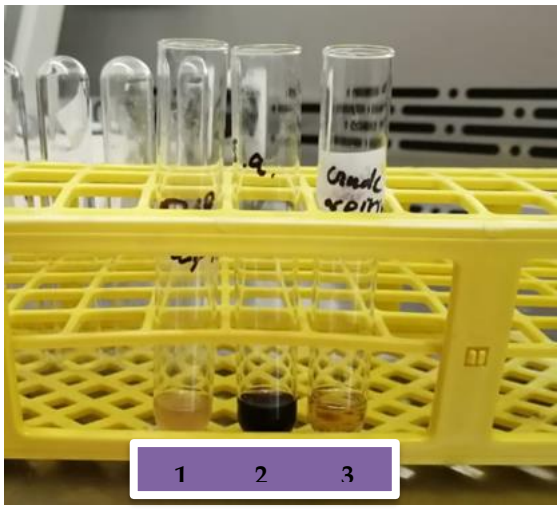

Figure 11: Result of ferric chloride test

The ferric chloride test indicates the absence of phenol in the tubes containing pure aspirin (1) and crude aspirin (3) but it indicates *an increased concentration of phenols in salicylic acid tube (2) showing the purity of our produced aspirin.*

6.3.1.2 Large scale production

The manufacture of aspirin has paralleled advancements in pharmaceutical manufacturing as a whole, with significant mechanization occurring during the early twentieth century. Now, the manufacture of aspirin is highly automated and, in certain pharmaceutical companies, completely computerized. In order to attend aspirin pharmaceutical manufacturing we must proceed first for a large scale production to avoid any problem that may apear as large production . So a static view and a dynamic view of pilot plant scale productio is presented below.(10)

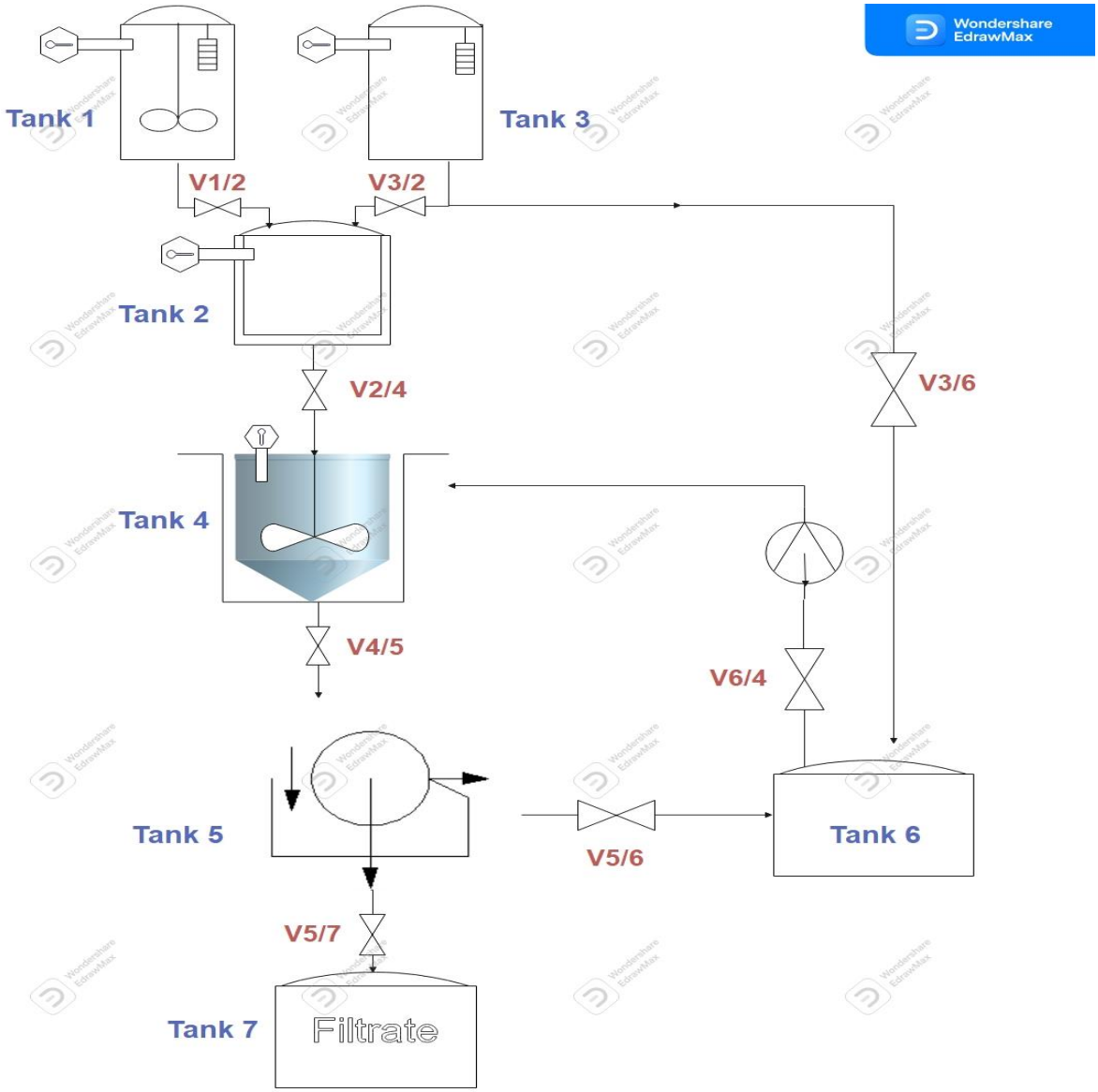

Figure 12:Static view of pilot plant scale aspirin production system

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Pilot Plant Scale for Aspirin Production

Introduction: Aspirin or Acetylsalicylic acid (ASA) is one of the first drugs to come into common usage, still widely used around the world with approximately 40,000 tonnes produced globally each year. Aspirin is an ingredient in many proprietary analgesic and cold/flu preparations. It is also used for the prevention of cardiovascular disease and there is growing work on its role in the prevention and management of cancer. Aspirin ($C_9H_8O_4$) remains on the World Health Organization's Model List of Essential Medicine (21st List 2019) both for its use in pain control and anti-platelet effects. Here we have to present a static and a dynamic view for the pretended aspirin bioreactor

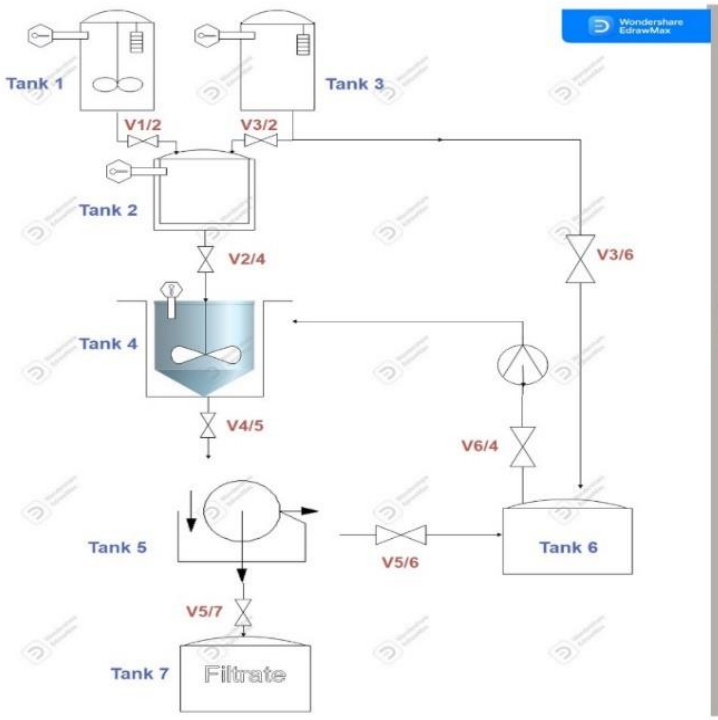

Figure 1 : An approximative view of aspirin system production

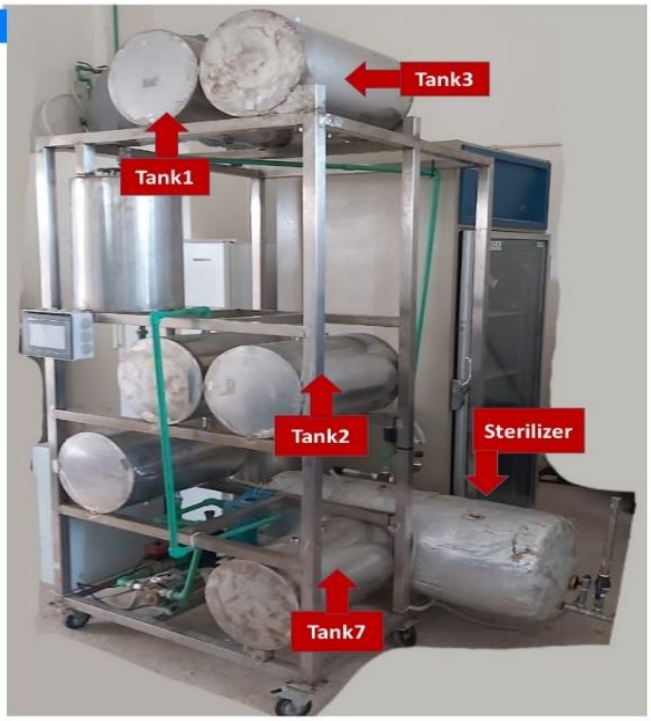

Figure 2 : The real Aspirin production system

1- Tank 1 :
- Place 1013.5g of salicylic acid
- Add 2.5L acetic anhydride
- Add 125ml concentrated H2SO4
- Mix and heat to 85° for at least 10min .

2- Tank 2 :
Open valve V1/2
- Cool down the mix to between 25- 28°.

3- Tank 3 to tank 2 :
Open Valve V3/2
- Add 5L warm water .

4- Tank 2 to tank 4
Open valve V2/4
- Cool the mix about 20min in an ice bath with mixing .
- Crystals are formed now.

5- Tank 4 to tank 5:
Open valve V4/5 and V5/6
- let the rotary drum vacuum filtration begin.
- Aspirin crystals are now in tank 6.

Open valve V5/7
- The filtrate is discarded out of the tank 7 .

6- Tank 6:
Open valve V3/6
- The obtained aspirin crystals are re-dissolved with 30L warm water for about 20 min.
- Here is the dissolved aspirin .

Open valve V6/4 and close valve V5/6

8- Tank 6 to 4
- The solution is pumped now from tank 6 to tank 4 for the second crystallization.

9-Tank 4:
- Re-cool the mix about 20 min in the ice bath with mixing.

Open valve V4/5 and V5/6
for the final rotary drum vacuum filtration.

10-Tank 5 :
- The filtrate is discarded into tank 7
- Crude Aspirin crystals are obtained in tank 6 and ready to be dry, tested and compressed for market delivery.

11- Sterilization:
Close all the valves
- The whole system must be sterilized by using sterilizer system

The valves are successively re-opened as mentioned as above.

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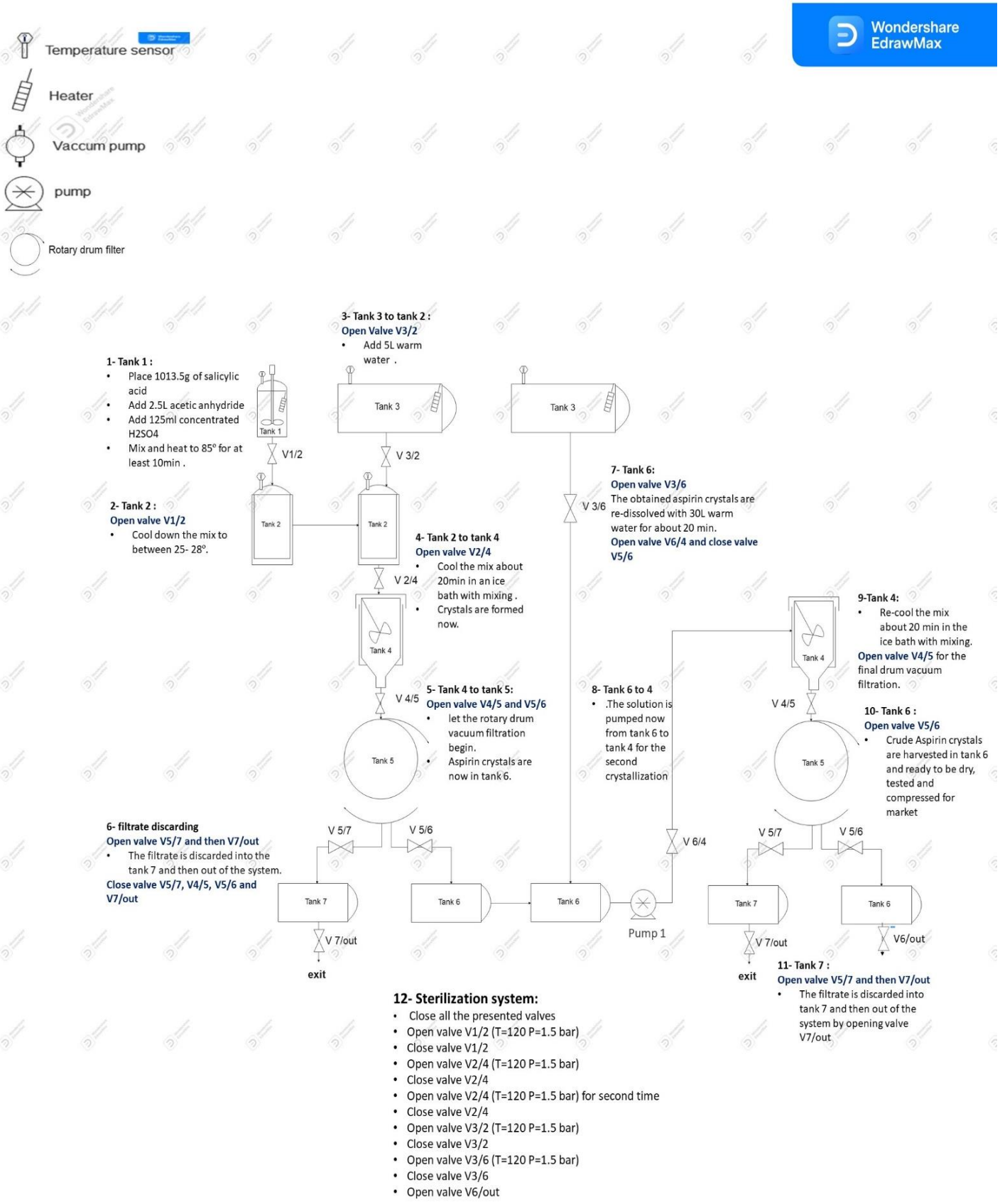

Figure 1 : Dynamic view of pilot plant aspirin production system

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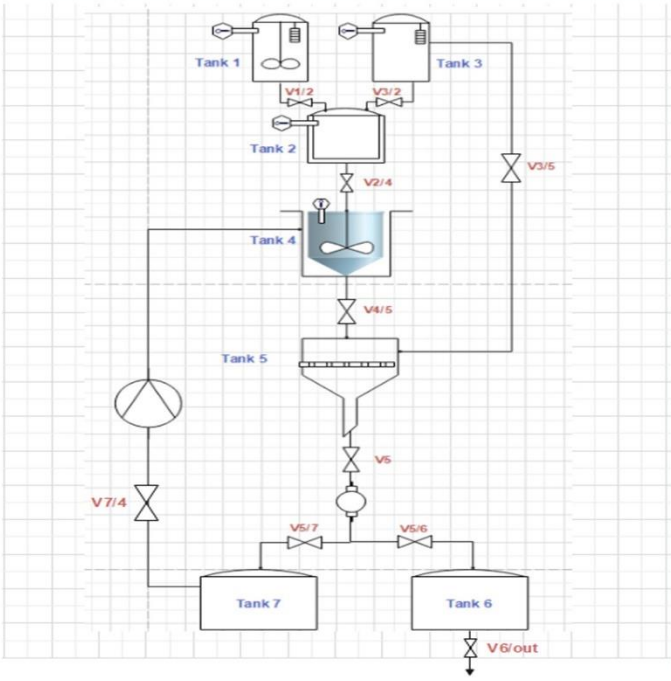
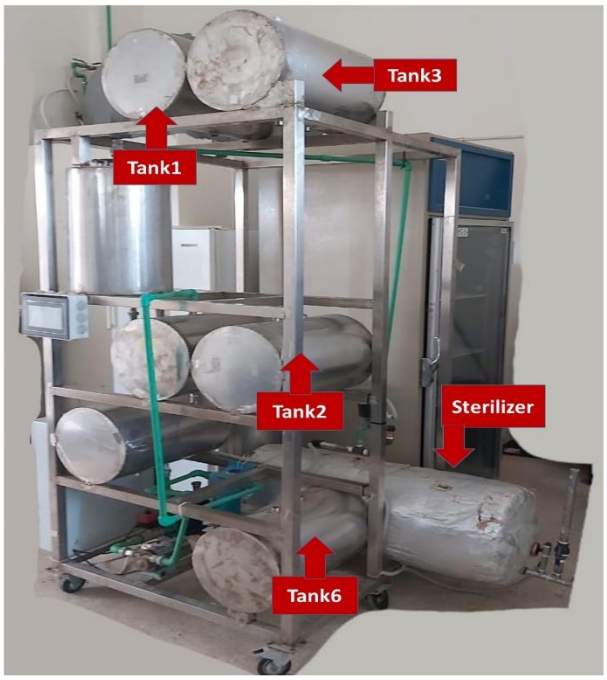


Figure 1 : An approximative view of aspirin system production



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Open Valve V3/2
- Add 5L warm water .

4- Tank 2 to tank 4
Open valve V2/4
- Cool the mix about 20min in an ice bath with mixing .
- Crystals are formed now.

5- Tank 4 to tank 5:
Open valve V4/5 V5 and V5/6
- let the vacuum filtration begin.
- Aspirin crystals are now into the filter paper in tank 5.

6- Filtrate discarding:
Open valve V6/out
- The filtrate is discarded from the tank 6 out of the system

Close valve V5, V6/out, and V5/6

7- Tank 5:
Open valve V3/5
- The obtained aspirin crystals are re-dissolved with 30L warm water for about 20 min.

Open valve V5 and valve V5/7

8- Tank 7 to 4:
Open valve V7/4
- The solution is pumped now from tank 7 to tank 4 for the second crystallization.

9-Tank 4:
- Re-cool the mix about 20 min in the ice bath with mixing.

Open valve V4/5 for the final vacuum filtration.

10-Tank 6 :
Open valve V5/6 and V6/out
- Crude Aspirin crystals are harvested from tank 5 and ready to be dry, tested and compressed for market.

11- Sterilization:
Close all the valves
- The whole system must be sterilized by using sterilizer system

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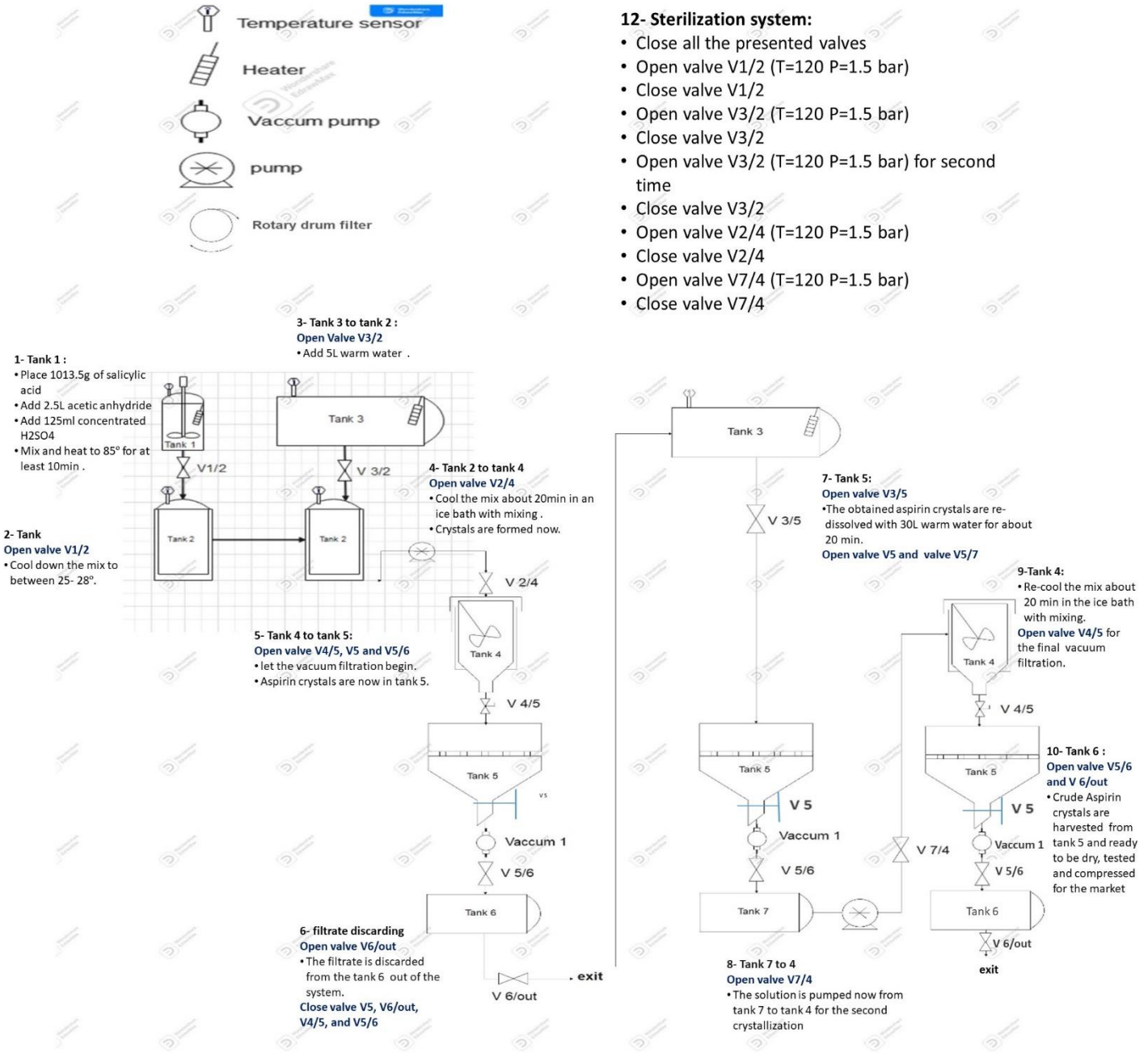

Figure 1 : Dynamic view of pilot plant aspirin production system

7 Part II: Penicillin

7.1 Overview

The term mold refers to filamentous microscopic fungi, some of which are of great economic and environmental interest. Some molds can have magnificent effects: processing of food raw materials, production of antibiotics, enzymes or flavoring agents etc... Others, however, are harmful: food alteration, causing mycoses and allergies and biosynthesis of mycotoxins.

Bacterial infections have been the main cause of death since antiquity (cholera, plague, tuberculosis...), but since 1928 the world has changed, finally we have a weapon against these bacteria, an antibiotic secreted by *Penicillium notatum* and *Penicillium chrysogenum,* called penicillin ...

Since 1940, antibiotic manufacturing has taken a predominant part in the pharmaceutical industry accounting for nearly 25% of its turnover. Penicillin and streptomycin and its derivatives are 60% of antibiotics(12).

7.1.1 Penicillium Genus:

This genus combines filamentous fungi belonging to the phylum of the *Ascomycetes*. This genus consists of approximately 227 species. The *Penicillium* are very common fungi in the environment, which can be responsible for many degradations. Their natural habitat is soil, food, organic matter, decomposing organic matter, compost, and cereals. They are common contaminants of temperate regions(12).

7.1.1.1 General cultural characteristics

The *Penicillium* grows quickly and easily on the culture media used routinely. They develop at moderate temperatures of the order of 20-27 ̊C. After 2 days of incubation, small, flat colonies, usually white, are observed. After 3-4 days of incubation, the sporulation will give the colonies their hue, most often in the tones green, blue green, gray green, yellow green, gray, blue, but also, for some species, yellow, orange, pink, or red. This color allows a first orientation in the identification of species: gray green for P. citrinum, P. cyclopium, P. italicum; yellow green for *P. chrysogenum*; dark green for *P. roquefortii*, *P. fellutatum*; pale yellow for P. *nalgiovense*; bright yellow to red for *P. pupurogenum*.. (13).

7.1.1.2 Penicillium chrysogenum

The *Penicillium chrysogenum* are filamentous fungi, mold-like. The branched conidiophore has a brush-like shape. Conidia are arranged in long chains. The thallus is green/white.

Like many other species of the genus *Penicillium, Penicillium chrysogenum* generally reproduces by forming conidiophoric brush-shaped spore chains (or conidia). Conidia are generally carried by air currents to new colonization sites. In *Penicillium chrysogenum,* the conidia are blue to blue-green and the mold sometimes gives off a yellow pigment. However, *Penicillium chrysogenum* cannot be identified on the basis of color, observations of morphology and microscopic characteristics are necessary to confirm its identity (14).

Penicillium chrysogenum is a very common species in soils, on organic matter, and food. This species can cause deterioration of textiles, papers, and food products.

Penicillium chrysogenum is a common fungus in subtropical temperate regions and can be found on food products (15), but it is mainly found in indoor environments, especially in damp buildings (16).

A study by Rodríguez-Sáiz et al. revealed that *P. notatum* does not produce as high amounts of penicillin as *P. chrysogenum*. Thus, *P. chrysogenum* was used industrially for the production of penicillin and xanthocillin X, for the treatment of infections and the production of enzymes: polyamine oxidase, phospho-gluconate dehydrogenase, and glucose oxidase (17),(18).

Improved strains of *Penicillium chrysogenum* have been used industrially to increase penicillin production. This improvement was achieved by: (1) overexpression of penicillin genes, amino acids of the penicillin precursor and some proteins, (2) increased peroxisome abundance, (3) increased NADPH via pentose phosphate pathway and cysteine biosynthesis, (4) increased carbon catabolism and energy production, (5) improved response to oxidative stress, (6) decreased virulence mechanisms (19).

7.1.1.3 Penicillin Antibiotics:

The antibiotic (from the Greek anti: counter, biotikos: concerning life) first used in 1889, with reference to a substance synthesized by one organism to destroy another, would later become more precise, as a chemical substance produced by a microorganism having the capacity to selectively inhibit growth or even destroy other microorganisms(12).

The antimicrobial potency of most classes of antibiotics is directed towards a unique characteristic of bacterial structure or their metabolic processes. The most common antibiotic targets are illustrated in fig 10 . The mechanism of action of antibiotics is as follows:

a) Inhibition of cell wall synthesis
b) Failure of the structure or function of the cell membrane
c) Inhibition of nucleic acid structure and function
d) Inhibition of protein synthesis
e) Blocking of key metabolic pathways.

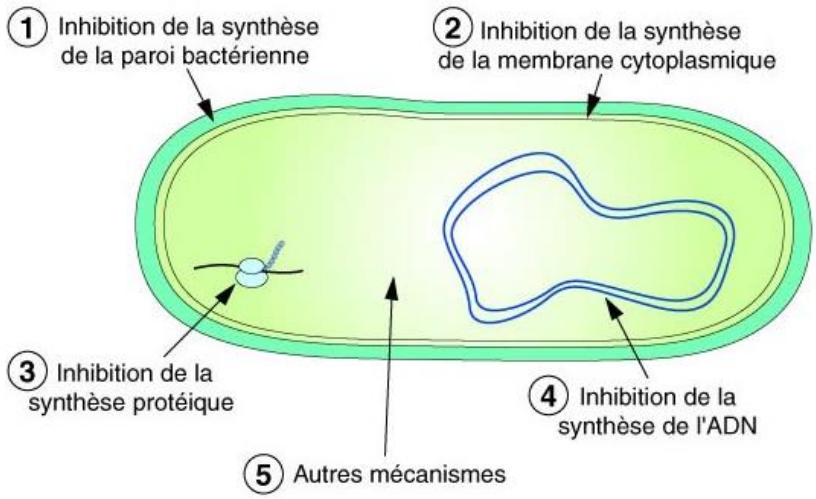

Figure 10: Bacterial cell and antibiotic target sites.

7.1.1.4 Chemical caracteristics

The chemical structure of penicillin is composed of a beta-lactam nucleus, a thiazolidine nucleus, and other side chains (Figure 11).

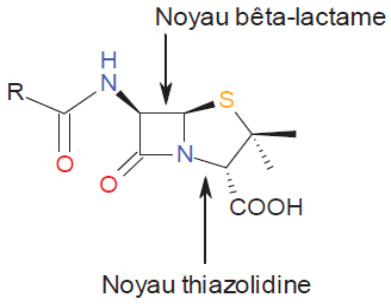

Figure 11: Structure of penicillin

Members of the penicillin class include: penicillin G, penicillin V, oxacillin (dicloxacillin), methicillin, nafcillin, ampicillin, amoxicillin, carbanecillin, piperacillin, mezlocillin, ticarcillin (Figure 12).

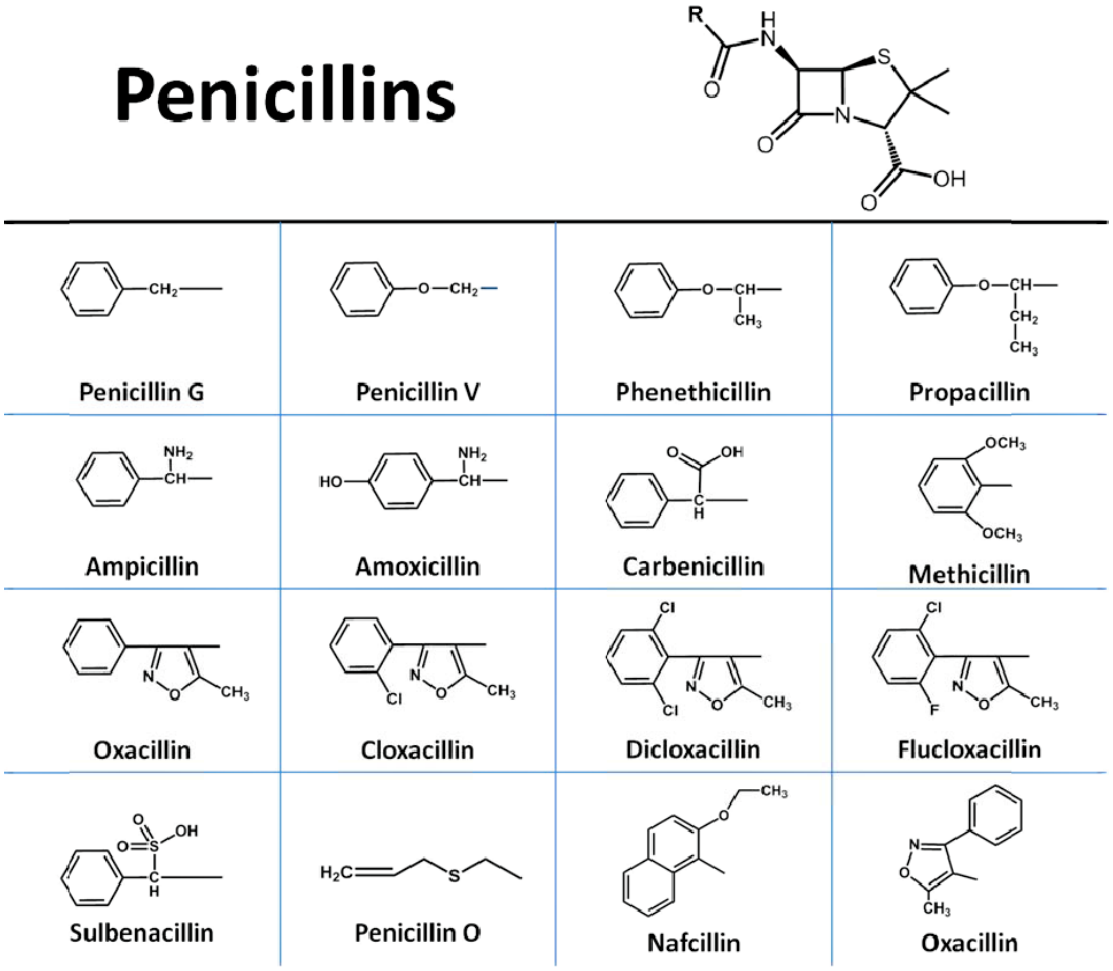

Figure 13 : Structure of penicillin class members (20)

7.1.2 Microorganism growth and penicillin production by P. Chrysogenum

Measurement of microorganism cell growth can be performed based on viable culturable cell count (UFC) or total cell count. It is possible to plot a growth curve that indicates the synchronized development of the cells as a function of time (Figure 13).

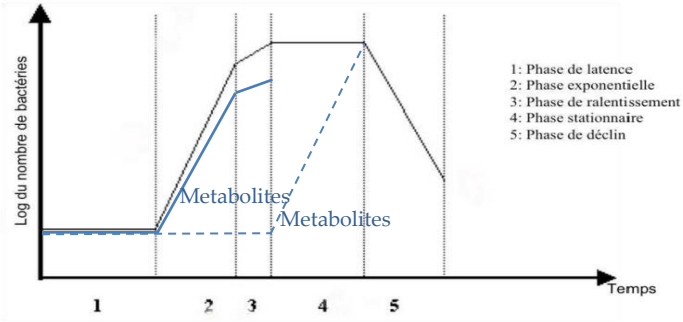

Figure 14: Change in the number of cells in the microorganism over time in days

The growth curve comprises 5 phases, more or less long, depending on the different microorganisms. (1) During the latency phase, the number of micro-organisms hardly increases because the cells do not divide. This is a period of adaptation of microorganisms to the environment. (2) The exponential phase is the time when microorganisms divide exponentially. It is in this phase that the rate of growth of the microorganisms is determined, and the primary metabolites are produced which are necessary for the survival of the microorganisms. (3) During the downturn, the growth rate is lower. (4) The stationary phase often reflects a balance between multiplication and cell death, the population stabilizes. For a large proportion of microorganisms, this time of growth is favorable to the production of secondary metabolites such as toxic compounds, antibiotics (e.g. penicillins G, V, and O produced by *P. chrysogenum*), enzyme inhibitors etc. (5) the phase of decline is due to nutrient depletion and waste accumulation. The number of micro-organisms that die exceeds the number of new micro-organisms from cell division (21).

When *P. chrysogenum* detects the presence of a carbon energy source preferred to lactose, it does not use lactose until the preferred substrate (e.g. glucose) is fully consumed (22). Once glucose is fully consumed, *P. chrysogenum* uses lactose which acts as a satisfactory carbon compound when used as a food source for the microorganism with a slow feeding rate, because lactose is a complex sugar. Thus, there is an intense synthesis of penicillin in this phase, due to lactose consumption (23) (Figure 14)

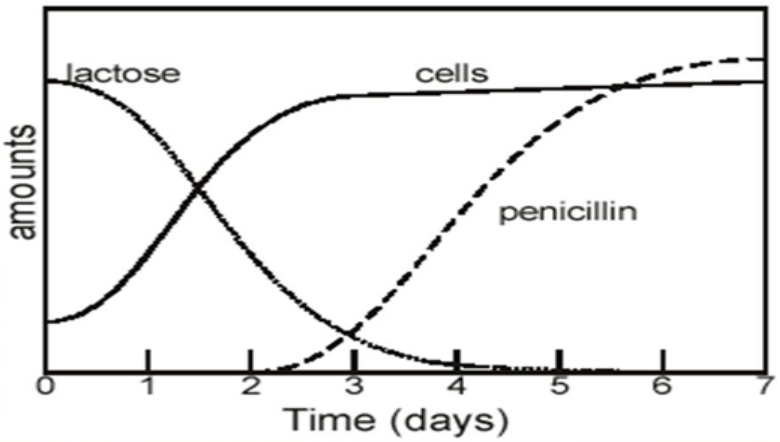

Figure 15: Variation in lactose, cell count, and penicillin over time (22)

7.1.3 Classification and spectrum of activity of natural penicillins

Currently, the name penicillin is used generically to refer to different molecules that have beta lactam structures and the same antibacterial activity as benzylpenicillin (penicillin G) - the original molecule extracted from *P. notatum*. The classification of

penicillins is based on chemical substitutions on the residue attached to the beta lactam ring, which confer different activities (Figure 15).

- Penicillin V or phenoxymethylpenicillin, has a narrower spectrum than penicillin G, it is active on:
  - Gram (+) shells: *Streptococcus pneumoniae*, *Streptococcus pyogenes*
  - Gram bacteria (+): *Corynebacterium diphtheriae*
  - Gram (-) bacteria: *Fusobacterium nucleatum*

- Penicillin O or almecillin may be useful in patients who are hypersensitive to penicillin G. It has a spectrum of activity very similar to penicillin G.

- Penicillin G or benzylpenicillin, is more active against Gram (+) bacteria and less active against Gram (-) bacteria:
  - Gram (+) shells: staphylococci, pneumococci, and other streptococci
  - Gram bacteria (+): *Bacillus anthracis*, *Clostridium perfringens*, and *Corynebacterium diphtheriae*
  - Gram (-) hulls: *Neisseria gonorrhoeae* and *Neisseria meningitides*
  - Gram bacteria (-): *Streptobacillus moniliformis* (24)

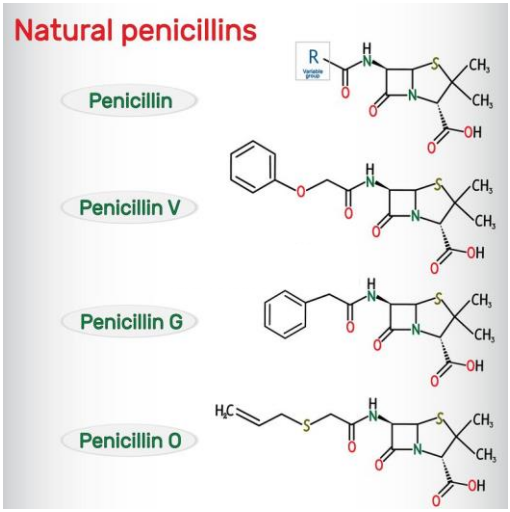

Figure 16: Natural penicillins

7.1.4 Diseases treated with penicillin:

Penicillin is the largest known family of antibiotics. It acts on bacteria in a targeted way, preventing their development or destroying them. It can fight bacteria that cause infections:

- Airways: like nasopharyngitis, bronchitis, sinusitis, pneumonia.

- Gastrointestinal tract: like listeriosis, acute diarrhea.
- Skin and soft parts: such as impetigo, skin abscess, cellulite (skin and tissue infection just below), erysipelas.
- Genital and urinary tract: such as cervicitis (cervical inflammation), urethritis, bacterial vaginosis (25).

7.1.5 Corrected protocol for penicillin G production:

Table 1: Constitutes of penicillin G fermentation broth(26)

Media	Percentage%
Lactose	3-4
Glucose	10
Corn steep liquor	4
CaCO3	1
Antifoam	0.25-0.5
Phenyl acetic acid	0.5
KH2PO4	0.4

Table 2: Parameters monitored in penicillin fermentation(26)

Parameter	Status
PH	Around 6.5-7
Temperature	26°C to 28°C
Aeration	continuous stream of sterilized air is pumped into it
Agitation	baffles which allow constant agitation

7.1.5.1 Penicillin purification and crystallization(27):

- First the filtrate is treated with H2SO4 solution to reach a pH between 1.5 and 2.2(Here the penicillin is presented ionized into the solution).
- Then an organic solvent must be added (Butyl acetate) (Here the ionized penicillin is present in the organic phase)

- To extract the penicillin from the organic phase an aqueous phase containing sodium or potassium ion must be added with the adding of NaOH to reach a pH from about 6 to 8
- Here the crystal of penicillin must be formed and then filtrated

7.1.5.2 Crystallization(28):

- From filtration, the penicillin rich solution is cooled to 5°C. As penicillin G only has a half-life 15 minutes at pH 2 at 20°C, this helps reduce enzyme and chemical degradation during the solvent extraction step.
- Crystals are highly organised inert matters. If grown without external interference, they grow in polyhedral shapes and exhibit many degrees of symmetry. Penicillin G is an odourless, colourless or white crystal, or crystalline powder. Crystallisation is essentially a polishing step that yields a highly pure product. It is done through phase separation from a liquid to a solid. To begin crystallisation, we must first have a supersaturated solution. Supersaturation refers to a state in which there are more dissolved solids in the solvent than can ordinarily be accommodated at that temperature at equilibrium. Supersaturation can be achieved usually by cooling, drowning, solvent evaporation, or by chemical reaction. Since the solubility of penicillin in its aqueous solution decreases with decreasing temperature, as the solution cools, its saturation increases until it reaches supersaturation and crystallization begins.

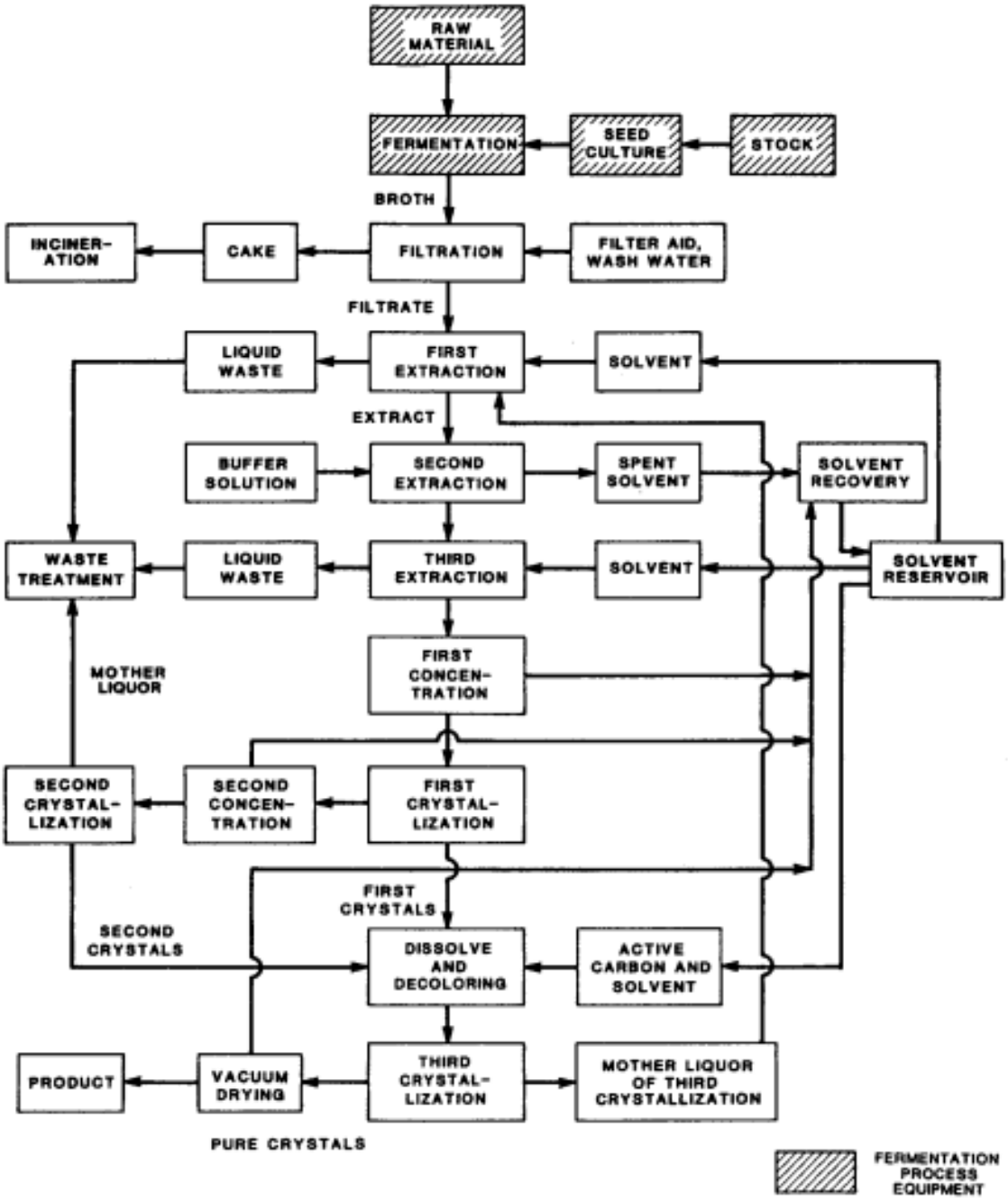

Figure 17: Block diagram for a general antibiotic production process(28).

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Lab Scale and whole Study of Pilot plant scale Production of Penicillin G With Penicillin Quantification

Introduction:
Penicillins (P, PCN or PEN) are a group of antibiotics originally derived from Penicillium molds (principally, P. chrysogenum, P. notatum and P. rubens). The discovery and manufacture of penicillins have changed the face of medicine. The several kinds of penicillin synthesized by various species of the mold *Penicillium* may be divided into two classes: the naturally occurring penicillins (those formed during the process of mold fermentation) and the semisynthetic penicillins (those in which the structure of a chemical substance—6-aminopenicillanic acid—found in all penicillins is altered in various ways).

Lab scale for Penicillin Production:

Preparation of agarose gel:

- We try to melt one tryptone tube by using a heated water (bain marie)
- We weigh 0.5 g of glucose powder
- In an erlenmeyer flask we mix the melted tryptone, the glucose and 10 ml of distilled water
- We heat them in a pressure cooker after boiling for 1 hour
- We let them cool down and we wait about 30 min until the gel are totally solidified
- We put them in the fridge until the time of microbial cultivation

Microbial culture:

- We cultivate the petri dish with the used strain of penicillium
- We incubated them at room temperature for 4 days

Preparation of liquid medium:

- We weigh 2 g of glucose powder, 0.8 g of lactose, 0.8g of peptone, 1g yeast extract, 0.15g of $MgCl_2$, 0.15g of KCl, and 0.1g of KH_2PO_4, 0.2g $CaCO_3$, 0.1g corn oil
- It is important to add penicillin precursor which called phenyl acetate at proportion 0.5%
- We add 100ml of distilled water
- We put the mixture in an erlenmeyer flasks
- We heat them with mixing for 15min by using a magnetic hot plate stirrer
- We autoclave the liquid medium, then let it cool down for about 30 min
- We inoculate it with the cultivated petri dish already prepared (about two or three colony)
- We incubate them at room temperature for 7 to 10 days with shaking

Isolation of pure colony:

- We took a colony from the cultivated dish and we isolate it into another tryptone medium to obtain a pure strain that will be used below.

Purification:

- Harvest liquid medium which contain penicillin by filtration
- Chill to 5-10°C (because penicillin is highly reactive and destroyed by alkali and enzyme)
- Acidify filtrate to PH 2.0-2.5 with H_2SO_4 (to convert penicillin to its anionic form)
- Extract penicillin from aqueous filtrate by adding ethyl acetate (at this very low PH as soon as possible)
- Discard aqueous fraction
- Allow the organic fraction to pass through charcoal to remove impurities and extract penicillin (this step is not important)
- Extract penicillin from ethyl acetate by adding 2% aqueous phosphate buffer (here the PB can be replaced by distilled water) at PH 7.5
- Acidify the aqueous fraction to PH 2-2.5 with mineral acid and re-extract penicillin into fresh ethyl acetate
- Add sodium bicarbonate to the solvent to crystallize the antibiotic as sodium salt
- Recover crystal in filter centrifuge or by filtration.

Pilot scale for Penicillin Production:

Preparation of liquid medium: (in the reactor)

- We weigh 400g of glucose powder, 100g of lactose(200g of skimmed milk powder), 160g of peptone, 200g yeast extract, 30g of $MgCl_2$, 30g of KCl, and 20g of KH_2PO_4, 40g $CaCO_3$, 20g corn oil
- Add penicillin precursor (phenyl acetate) proportion 0.5% (100 g)
- we innoculate the bacterial colonnies from the petri dish already prepared
- We add 20L of distilled water
- We incubate them at room temperature for 7 to 10 days withn shaking (moto shaking) and control the PH between (6.5-7)

Purification:

- **after the incubation period (10days)** we filter the liquid nedium by adding butyl acetate= 1714ml (proportion 14/2 → 14 ml filtrate need 2 ml butyl acetate)
- **penicillin dissolved in butyl acetate**
- The centrifugation method was applied to eliminate the pellet containing cells debris and all other contaminant (4000x for 15 min)
- We add 600g of sodium bicarbonate for the supernatant to obtain the penicillium in salt form.

	REACTIF	COST
Liquid medium	Glucose powder	2.8$
	Milk powder	2.75$
	Peptone	4$
	Yeast extract	63$
	$MgCl_2$	2$
	KCl	3$
	KH_2PO_4	4.2$
	$CaCO_3$	14.7$
	Corn oil	0.1$
	Phenyl acetate	28$
	Distilled water	3$
Purification	Butyl acetate	**3.5$**
	Sodium bicarbonate	**75$**
Total	**206$**	

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بسم الله الرحمن الرحيم

AECENAR
Association for Economical and Technological Cooperation in the Euro-Asian and North-African Region

CONDITIONS OF PENICILLIN PRODUCTION

Introduction:

Penicillin G is a broad-spectrum, beta-lactam naturally occurring penicillin antibiotic with antibacterial activity. Penicillin G binds to and inactivates the penicillin binding proteins (PBPs) located inside the bacterial cell wall. Inactivation of PBPs interferes with the cross-linkage of peptidoglycan chains necessary for bacterial cell wall strength and rigidity. This interrupts bacterial cell wall synthesis and results in the weakening of the bacterial cell wall and eventually causing cell lysis.

1. Temperature :

According to the results of chromatograms obtained in different temperature conditions, temperature variations cause changes in the rate of penicillin G production in P. chrysogenum medium. The highest rate of production was in 28˚C. The production rate of penicillin was reduced significantly at temperatures above 30 ° C.

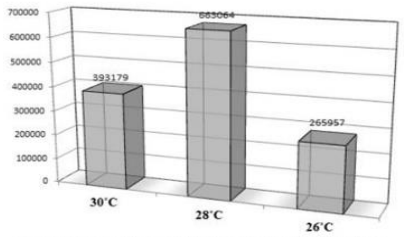

Figure 1: Comparison of obtained results from injection of extractions in different temperature conditions

2. pH :

Present-day penicillin fermentations are highly automated and computerized. All the necessary precursors, ammonia, sugar, carbon dioxide, oxygen are controlled, with thorough monitoring of temperature and pH for optimal antibiotic production. The pH should be between 6.4 and 6.8 during the active production phase.

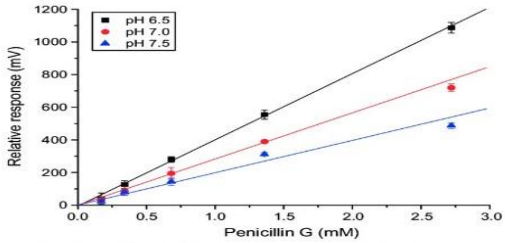

Figure 2: Penicillin G calibration curves at various buffer pH values

3. Incubation time :

Highest rate of antibiotics produced by P. chrysogenum was from 6-8 days after its cultivation. Before the fifth day, the fungus can not produce growth inhibitor substances. Therefore, the duration of fungal cultivation is effective on production of penicillin.

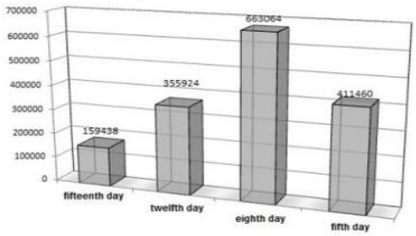

Figure 3: Comparison of obtained results from injection of extractions with different duration of cultures

4. Yeast extract quantity:

After comparing the results of chromatograms which were obtained from injections in different conditions, optimum value of medium components was determined as: 3 g yeast extract in one liter of pure distilled water.

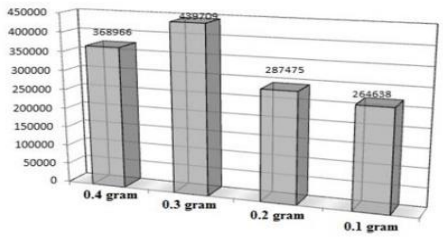

Figure 4: Comparison of obtained results from injection of extractions from isolate 2031 media with different levels of yeast extract

5. Shaker movement :

The results of chromatograms which were obtained from injections in different conditions or the optimal amount of Shaker movement was set at 120 rpm.

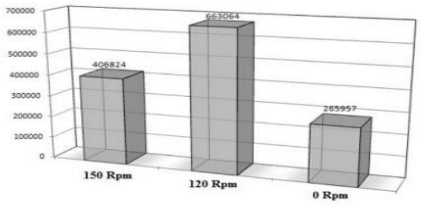

Figure 5: Comparison of obtained results from injection of extraction from isolate 2031 media located on different rotates of Shaker

6. Methods of extraction :

In comparison of different methods of extraction using different solvents, the highest rate of penicillin extraction was by using butyl acetate solvent and a three-step method.

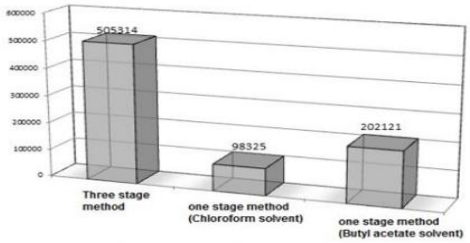

Figure 6: Comparison of obtained results from different extraction methods

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7.2 Materials and methods:

7.2.1 Strain collection

A strain of *Penicillium chrysogenum* has been preserved at the Doctoral School at the Lebanese University in Tripoli (Figure 16).

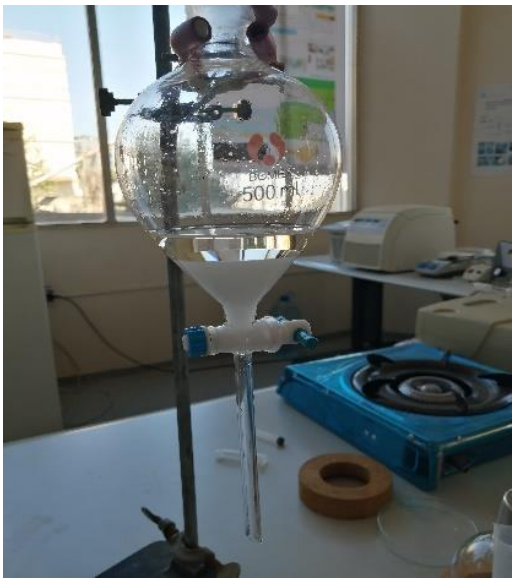
Figure 18: Penicillium chrysogenum

7.2.2 Experiment 1: Ethyl acetate preparation:

- 100 ml ethanol with 100 ml spirit of vinegar (concentrated vinegar) are added in a round flask
- Then 20 ml of concentrated H_2SO_4 is added (added slowly)
- The flask is connected to a water cooled reflux condenser
- The mixture is heated under reflux for 30 min on round flask heater

- After 30 min the flask is cooled
- Now a separation is carried out to purify the produced ethyl acetate of any excess acid
- In a separating funnel containing the produced ethyl acetate we add 10g of anhydrous sodium carbonate diluted in 50 ml distilled water.
- Shacking- Degassing- waiting 2 minutes- separation (we keep the organic phase into the funnel)

- Then we add 10g of anhydrous calcium chloride diluted in 50 ml distilled water.
- Shacking- Degassing- waiting 2 minutes- separation (we keep the organic phase into the funnel)



- Finally, we carry out to a final distillation of the obtained organic phase for about 10 min to obtain the purified ethyl acetate

- To ensure the purity of ethyl acetate and the absence of acid we test a little of our product on sodium bicarbonate, there must be no effervescence.

7.2.3 Experiment 2: Penicillin production

- The same steps already mentioned in a last report are followed(29).

7.2.3.1 Penicillin purification:

To purify the produced penicillin we had pursued different three protocol.

The first (P1) without using charcoal (just: filtration-ethyl acetate-centrifugation-sodium bicarbonate)

The second by using charcoal and V/V from the ethyl acetate

The third by using charcoal and V [14:2] from the ethyl acetate (for each 14 ml of the filtrate use 2 ml of ethyl acetate)

Then we have tested the filtrate when we had obtained a lot of contaminant.

For this reasons the protocol was retried with the autoclaving of the liquid medium before adding the *penicillium* colony. Then the quantification will be tried.

7.2.4 Experiment 3:Second trial for penicillin production (12)

7.2.4.1 Preparation of the medium and preparation of the inoculum

Preparation of the liquid medium (Broth): 0.5 g peptone, 0.1 g tryptone, 0.1 g glucose, 0.1 g yeast extract, 0.25 g NaCl, 50 ml distilled water were mixed and heated in a beaker. The pH was measured and adjusted to 7 by adding a few ml of H_2SO_4.

Broth description: Broth cultures are **a method of growing bacteria in a liquid growth medium**. They're used to grow and maintain cultures for a laboratory. Different bacteria may grow differently in broth cultures. They are: Aerotolerant anaerobic bacteria evenly dispersed throughout the culture.

The medium was then placed in 4 tubes, autoclaved for 1 hour and then cooled.

An inoculum is taken from the strain *Penicillium chrysogenum* using a sterile loop and then transferred and mixed in the liquid medium. The tube is incubated at room temperature for 4 days.

7.2.4.2 Culturing

Two nutrient media were prepared in 100 ml of distilled water for each experiment (Figure 17).

Medium 1: 2g glucose, lactose (2.7g for experiment 1; 0.8g for experiment 2), 1g yeast extract, 0.5g corn oil, 0.8g peptone, 0.1g KH2PO4, 0.15g MgCl2, 0.2g CaCl2, 0.2g $Na2CO_3$, 0.8ml phenyl acetate (in experiment 2 only)

Medium 2: 10g glucose, lactose (10.7g for experiment 1; 3g for experiment 2), 4g peptone, 1g CaCl2, 1g $Na2CO_3$, 0.4g KH_2PO_4, 0.8ml phenyl acetate (in experiment 2 only)

The pH of the 2 media was measured and then adjusted to 7 by adding a few ml of H_2SO_4.

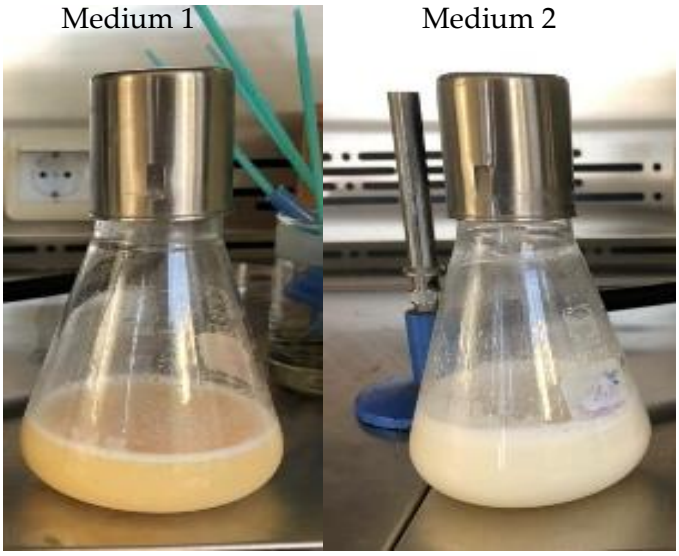


Figure 19:Medium 1 left and medium 2 right

10 ml of each of the 2 mixtures were then added to a test tube each. The broth was then mixed and 2 ml were added to the 2 tubes containing medium 1 and 2 so that *P. chrysogenum* could adapt to the medium. Finally, the contents of the tubes were returned to the 2 Erlenmeyer flasks containing media 1 and 2 and they were incubated at 26 °C with stirring for 10 days.

Figure 20: Incubation of the two medium at room temperature for 10 days

7.2.4.3 Filtration

	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7	Tube 8	Tube 9
Concentration of commercial penicillin G (IU)	1 00 000	10,000	1,000	100	30	25	20	16	10
Penicillin G volume (ml)	9	9	9	7	3	5	5	4	2.5.
Volume of NaCl (ml)	1	1	1	1	7	1	1	1	1.5.

Filtration is a technique that involves passing a liquid through a filter whose pores have a diameter of 0.2 μm. The microorganisms are too large and are therefore retained by the filter. After filtration, the pH of the filtrate was adjusted to 2-2.5 in order to stop any reaction that could degrade penicillin. The filtrate was then kept cold.

7.2.4.4 Sensitivity test

This step was carried out in experiment 2 only.

A *Staphylococcus aureus* bacterium has been preserved at the Doctoral School, Lebanese University, Tripoli.

A solid medium (agar gel) was prepared. A few ml of the filtrates were then poured into the petri dish and *S. aureus* was cultured. The box was incubated at 37 ̊C for 24 hours.

7.2.4.5 Purification

3 ml of each filtrate were added with 3 ml of butyl acetate which serves as solvent for separating the organic phase and the aqueous phase using a separating funnel. The organic phase was recovered in a tube and a few ml of $NaSO_4$ were added in order to carry out the crystallization. The tube was kept cold for 24 hours for the crystallization step to be completed. Finally, the contents of the tube were filtered and dried in order to collect penicillin in powder form.

7.2.4.6 Quantification

Dilution was carried out in 9 test tubes containing NaCl and commercial penicillin (1,000,000 IU) as shown in Table 3:

Table 3 : Dilution of commercial penicillin

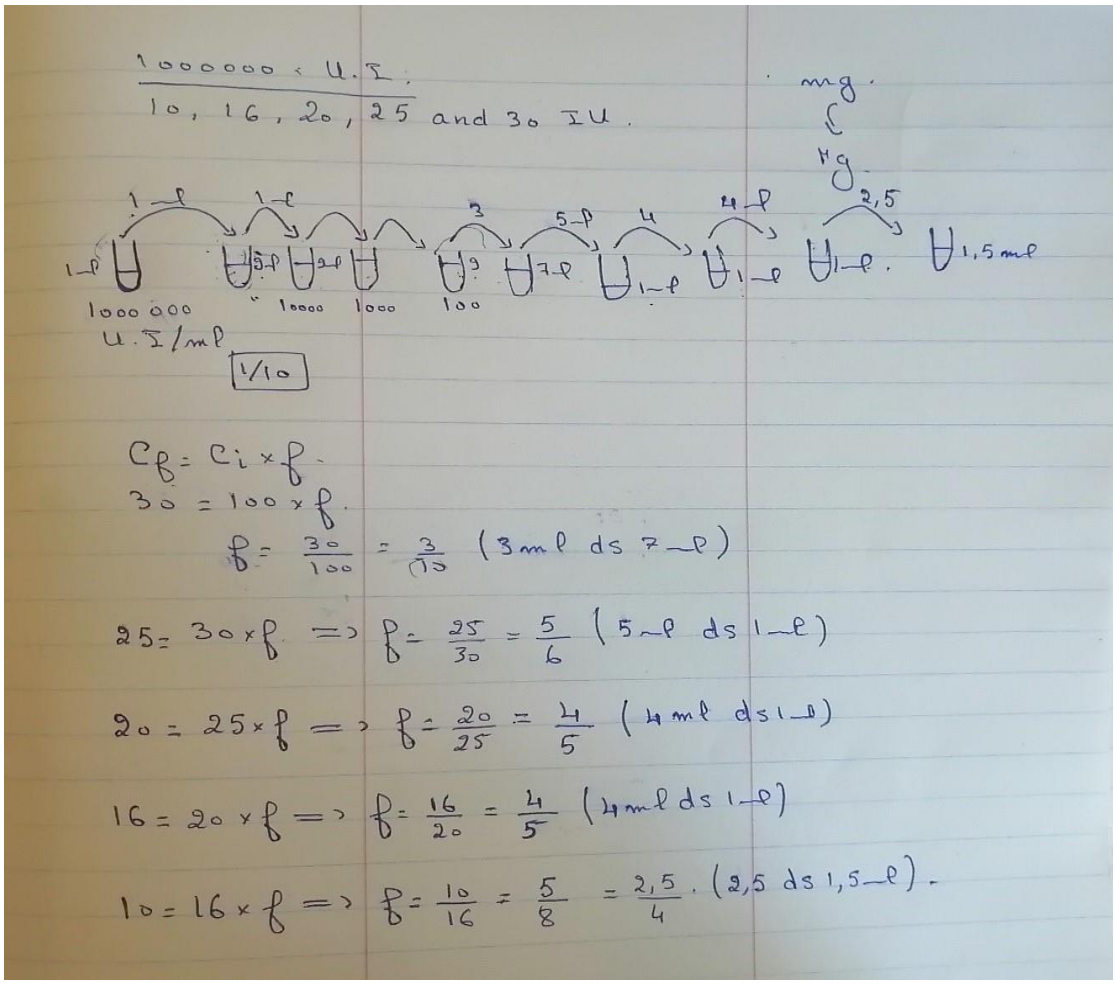


Figure 21: serial dilution for penicillin quantification

The amount of penicillin produced from the 2 filtrates was dissolved in a phosphate buffer and filtered to get rid of the impurities. Then, a colony of S. aureus was dissolved in 5 ml NaCl, then NaCl was continued until its turbidity became similar to that of the standard (0.5 McFarland) (Figure 21).

The Mueller-Hinton medium was prepared in a petri dish. A swab was immersed in the tube containing S. aureus and inoculated into the petri dish. An antibiogram was carried out by applying 20 µl of each antibiotic concentration (30, 25, 20, 16, 10 UI) to the disks present in the petri dish and then incubated for 24 hours.

0.5 McFarland Standard

Figure 22: McFarland turbidity test

7.3 Results and discussion

7.3.1 Experiment 1: Ethyl acetate preparation:

We had obtained about 80 ml of purified ethyl acetate.

Figure 22: ethyl acetatate showing any effervescence with sodium bicarbonate

7.3.2 Experiment 2: Penicillin production

Here their is any inhibition mentionned against E.coli so the penicillin production is not succeed(Fig.22)

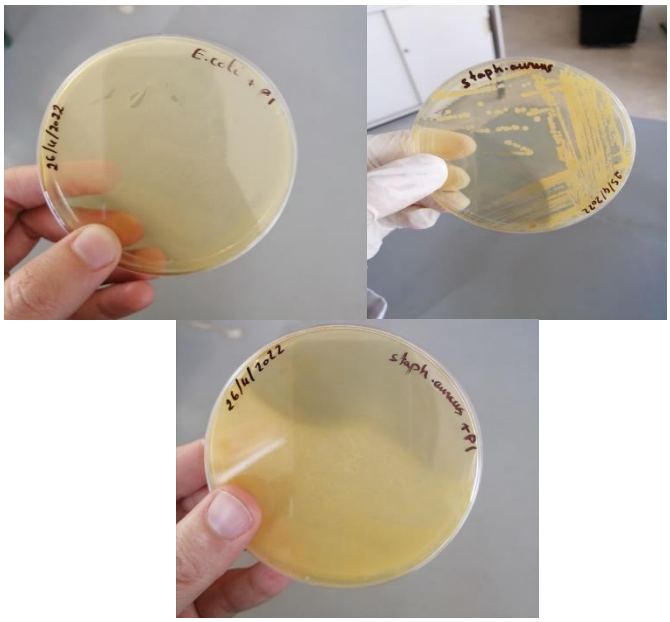


Figure 23: Result of quantification test showing any antibacterial activity against E.coli

7.3.3 Experiment 3: Second trial of penicillin production

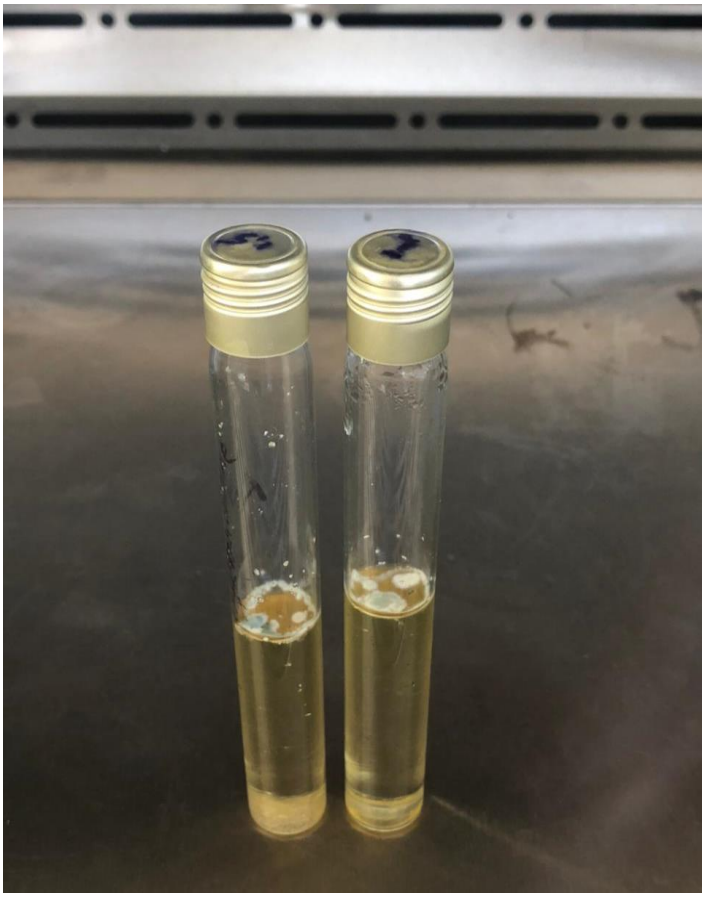


Figure 24: broth medium after 4 days of incubation with penicillum Chrysogenom

The filtrate 1 and 2 antibiograms showed that different concentrations of commercial penicillin caused inhibition of bacterial growth by the formation of the inhibition zone. However, there is no zone of inhibition at the disk corresponding to the penicillin G

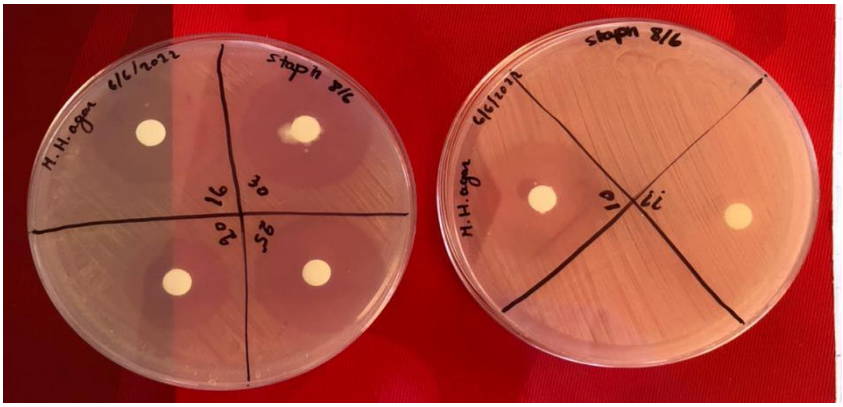


Figure 25: Antibiogram showing results of filtrate 2 quantification

produced, which indicates that our experiment has failed (FIG. 25 and 26).

A sensitivity test was performed prior to the quantification step to investigate the presence or absence of penicillin G. Figure 27 shows the growth of the bacterium thus confirming the absence of penicillin G.

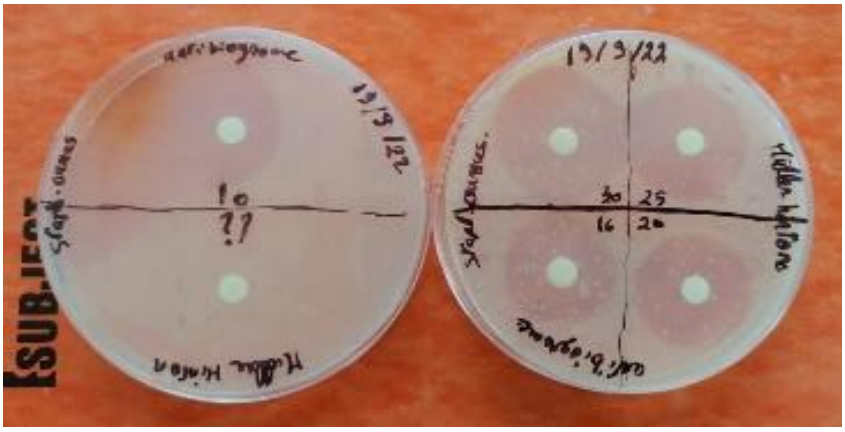


Figure 26: Antibiogram showing the results of the quantification of filtrate 1

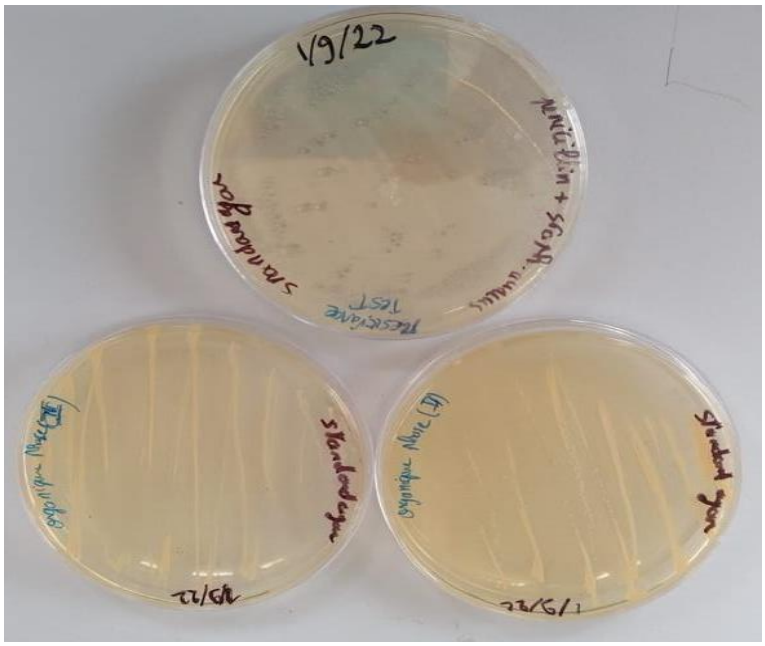


Figure 27: Result of sensitivity test of experiment 2. (a) control, (b) filtrate 1, (c) filtrate 2

7.3.4 Discussion

Our work focused on the cultivation of *Penicillium chrysogenum* on a liquid medium, producing and quantifying penicillin G by conventional methods. In the presence of glucose and lactose in the nutrient broth, glucose is preferentially consumed [first] by *P. chrysogenum* since it is considered to be the most important energy source. Lactose is consumed secondarily in a more difficult way, thus inducing the production of

penicillin. In the first experiment, lactose was greater than glucose in both media, inhibiting penicillin formation. According To (23) The presence of a high amount of lactose inhibits the production of penicillin G. In addition, phenyl acetate, the precursor that leads *P. chrysogenum* to produce penicillin G, was absent in our first test. Yet, (23) stresses that it is a key product during fermentation for the production of Penicillin G.

In the light of the 1st experiment, the amount of lactose was significantly less than that of glucose in our 2nd test. Also, in the 2nd experiment, 0.8 ml of the phenyl acetate precursor was added once in order to direct *Penicillium chrysogenum* to produce penicillin G only. (23)**.** However, the phenyl acetate precursor is the best precursor used to date, but it is preferable to add it several times in small amounts during fermentation in order to avoid toxic effects and thus improve the results of the experiment, but this has not been achieved in our experiment. However, in all cases, the production of penicillin G failed, suggesting that the laboratory temperature was not suitable because of the sunlight that affected both mixtures of the incubator causing the destruction of *Penicillium chrysogenum*. In addition, for greater precision in the production of penicillin, HPLC or TLC techniques could be used prior to quantification by the antibiogram to determine the presence of penicillin G and other active products that may be present(13). However, these 2 analytical methods are commonly used in laboratories for the separation and rapid identification of the constituents of a given extract.

8 Part III: Ampicillin

8.1 Overview

8.1.1 Discovery: (30)

In 1957, the isolation of 6-aminopenicillanic acid from penicillin fermentations led to the production of many semi-synthetic penicillins, of which ampicillin is the more widely used. In 1961, 1-amino-benzylpenicillin, known as ampicillin, was marketed .

8.1.2 Chemical Definition and Structure: (30)

Ampicillin (AMP) is a semi-synthetic, aminopenicillin-family, elasticated lactam antibiotic with a broad spectrum of action in the prevention and treatment of various bacterial diseases. It is one of the most important anti-kinetic and broad-spectrum penicillins used today that act on Gram-positive bacteria and some Gram-negative bacteria, but is inactivated by penicillinases, a class of enzymes that inactivate penicillin by hydrolysis of the kinetic-lactam ring.

The chemical structure of ampicillin is illustrated in the following figure:

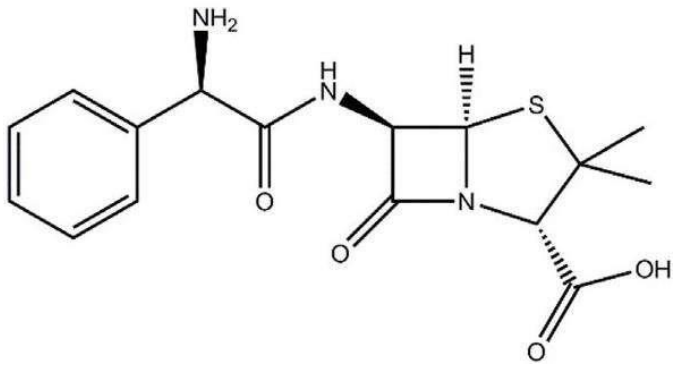


Figure 28: Chemical Structure of Ampicillin

8.1.3 From penicillin to ampicillin: (30)

Although penicillins differ in their lateral chain structure, they all have the same antibacterial activity as benzylpenicillin or penicillin G, the original molecule extracted from *P. notatum*. Benzylpenicillin is narrow-spectrum, only Gram-positive bacteria (streptococci) and some Gram-negative bacteria such as *Treponema pallidum* are susceptible to it as a causal agent for syphilis and meningococci. The inability to act against Gram-negative bacteria is observed not only among benzylpenicillins, but also among many different antibiotics. This is due to two factors: firstly, unlike Gram-positive bacteria, Gram-negative species contain the outer membrane, which acts as a selective barrier, blocking the penetration of penicillin; secondly, some Gram-negative bacteria have acquired specific genes that code for penicillinases. Given this, some antibiotics such as ampicillin, carbenicillin and amoxicillin have been developed semi-synthetically with different side chains. These side chains give antibiotics the ability to

escape the degradation capacity of certain enzymes produced by certain bacterial strains and to facilitate the movement of antibiotics through the outer membrane of these bacterial cell walls. This dual-component capability increases their spectrum of activity against Gram-negative bacteria.

Ampicillin is distinguished from penicillin G or benzylpenicillin by the presence of an amine group that helps to increase the hydrophilicity of ampicillin, allowing it to diffuse through the porins in most external membranes of Gram-negative bacteria. As a result, ampicillin has a broad spectrum of action, being active against non-lactamase-producing Gram-positive and Gram-negative bacteria, enzymes produced by microorganisms as a mechanism of resistance to and capable of hydrolyzing the antibiotics, namely *N. gonorrhoeae, N. meningitidis, H. influenzae, T. pallidum, Leptospira spp., E. coli, Salmonella spp., Shigella spp.* and *Proteus mirabilis*).

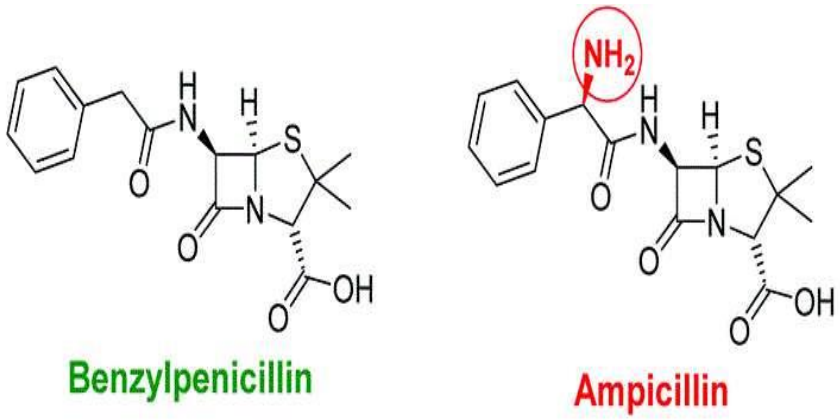


Figure 29: Structural relationship between penicillin G and its semi-synthetic antibiotic ampicillin

8.1.4 Therapeutical uses: (30)

- Respiratory tract infections:
- Bacterial meningitis:
- Sepsis and endocarditis
- Genitourinary tract infections:
- Gastrointestinal infections:

In addition, the spectrum of activity of ampicillin can be extended and improved by co-administration with inhibitors of spective lactamases, such as clavulanic acid, thereby allowing ampicillin to be effective against penicillinase-producing bacteria (such as *H. influenzae, M. catarrhalis* and *Bacteroides fragilis)*; or sulbacam, which is also an inhibitor of beta-lactamases.

8.1.5 Synthesis pathways: (30)

Only two antibiotics, penicillin G and penicillin V, used in clinical practice are products of a fermentation process and are therefore considered natural molecules. The majority of the antibiotic-Lactams used in clinical practice are classified as semi-synthetic because the fraction of Lactam is obtained from the enzymatic hydrolysis of a natural fermentation product; penicillin G or penicillin V, while the acyl side chain is obtained by chemical or chemo-enzymatic synthesis:

8.1.5.1 Chemical Synthesis:

Chemical coupling of the alkyl-lactam moieties with an acyl side chain has dominated the industrial production of semi-synthetic alkyl-lactam antibiotics since their discovery. Ampicillin can be produced by chemical synthesis which can reach yields as high as 90% , but this technique generally involves expensive steps, such as temperatures as low as -30° C., the use of large volumes of toxic organic solvents (exp: methylene, dichloromethane . . . .) and the use of highly reactive compounds (exp: pivaloyl chloride). Despite recycling solvents and auxiliary reagents where possible, this technique still generates a large amount of non-biodegradable waste.

8.1.5.2 Biocatalytic synthesis:

The coupling of the crystals of the LETLAM with the acyl side chain can be carried out enzymatically using penicillin G acylase (PGA). A less studied enzyme, the acetate-amino ester hydrolase (AEH), may also be used for this reaction when the acyl side chain has an amine group in position 1. Furthermore, the coupling can be carried out under thermodynamic control, which uses an unactivated acyl side chain; or under kinetic control, which requires an activated side chain (typically an ester or an amide). Noting that thermodynamically controlled synthesis, also called direct synthesis or equilibrium controlled synthesis, is only capable of achieving minimum yields (1% or less) in aqueous medium. Under typical reaction conditions, the substrates are in their ionized forms, which PGA does not accept. Thermodynamically controlled synthesis is then unnecessary for the synthesis of semi-synthetic Lactamine-based antibiotics.

8.1.5.3 Comparaison of synthesis techniques: (30)

The obvious disadvantage of the enzymatic coupling process compared to the chemical coupling process is the lower yield of the product. However, enzymatic coupling has undeniable advantages over chemical coupling in terms of raw material cost, environmental impact, product quality and ease of use; because it is carried out at ambient temperature, pressure and pH; and does not require toxic or dangerous reagents or solvents.

One-pot synthesis of ampicillin:

Cascade conversions, which combine several reactions without intermediate recovery steps, are increasingly being studied to make syntheses more respectful of the environment is economically advantageous. The replacement of a multi-stage synthesis requiring

intermittent isolation by a cascading process eliminates the need for isolation and purification of intermediates and thus results in smaller reactor volumes, shorter cycle times, higher volumetric and spatio-temporal yields and a reduction in the amount of waste generated. Cascade conversions may combine several biocatalytic steps, several chemocatalytic steps, or may combine both biocatalytic and chemocatalytic steps. In general, it is easier to combine several biocatalytic steps because most enzymes have similar operating conditions.

In this work, a cascade conversion was carried out with two biocatalytic reactions in an entirely aqueous medium to synthesize ampicillin. In the first reaction, 6-APA containing the core of the ß-LACTAM was produced from the hydrolysis of penicillin G using immobilized PGA (iPGA). The by-product of this reaction, phenylacetic acid (PAA), is a known inhibitor of iPGA. In the second reaction, ampicillin was produced by a **kinetically controlled** coupling of 6-APA and the (R)-phenylglycine methyl ester side chain, catalyzed by immobilized PGA (iPGA), (AEH could also be used in this 2nd reaction).

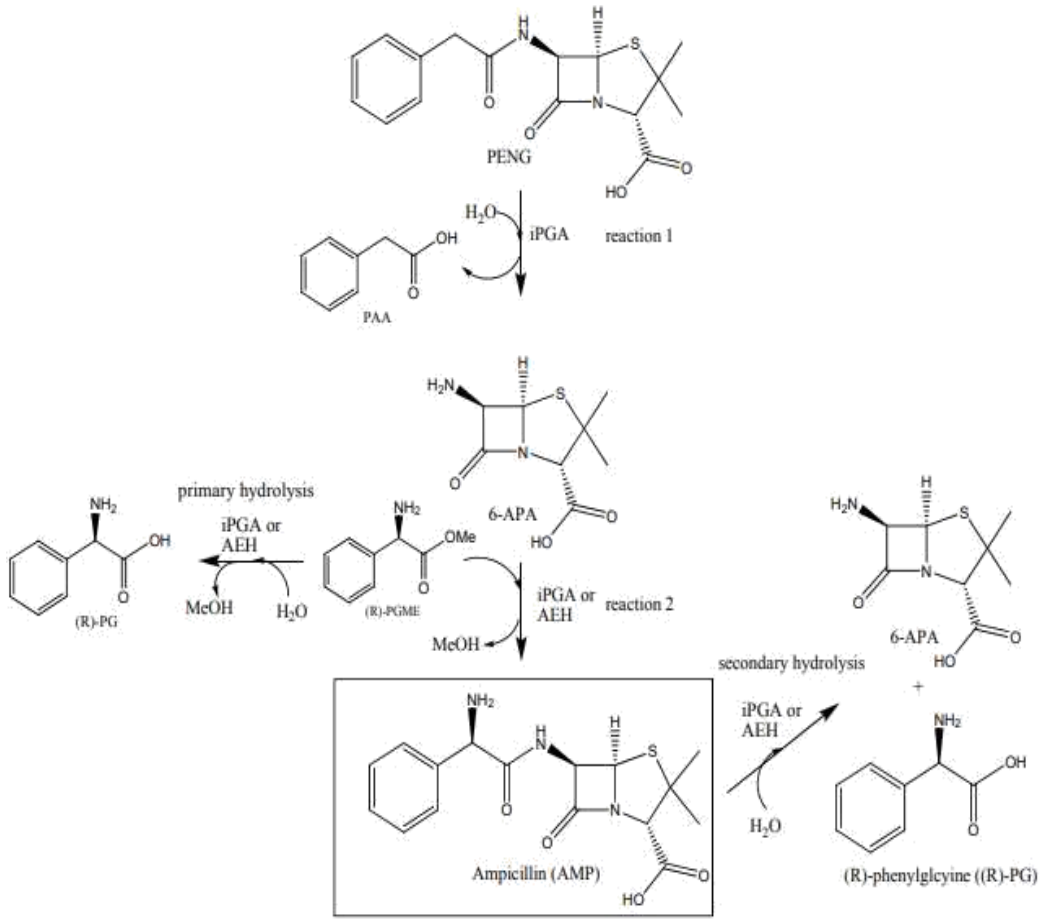


Figure 30: Direct two-enzyme, one-pot conversion of penicillin G (PENG) to ampicillin (AMP) using iPGA and AEH.

Penicillin G acylase (PGA) can act as a hydrolase as well as a transferase, which means that this same enzyme catalyzes the synthesis of ampicillin as well as the hydrolysis of the activated acyl donor R-PGME and the hydrolysis of the newly formed antibiotic. The low yield can thus be attributed mainly to the primary hydrolysis reactions of R-PGME and secondary hydrolysis of the ampicillin produced, which are catalyzed by iPGA. Since AEH enzymes are unique in their specificity to the acyl moiety with respect to the 1-amine groups, they cannot then catalyze the hydrolysis of penicillin G to obtain 6-APA and they are not inhibited by the PAA, hence their advantage in this cascade.

In addition, there are two methods for the synthesis of ampicillin in one pot which can be proposed:

One-pot synthesis, 1 step (1P1S):

This is a batch process. Penicillin G, (R)-PGME, and all enzymes (either iPGA or both iPGA and AEH) are added to the reaction and the reaction may continue.

One-pot synthesis, two steps (1P2S):

Following which penicillin G and iPGA are added to the reaction and the hydrolysis reaction can proceed almost completely to produce 6-APA. When the hydrolysis reaction is nearly complete, (R)-PGME and either AEH or additional iPGA are added to the reaction mixture.

However, the one-pot two-step (1P2S) synthesis produces a higher overall yield.

Based on these data, the ampicillin synthesis technique chosen in my present study is **one-pot, two-stage** (1P2S) **synthesis**.

8.1.6 Bacterial strains those will be used in our trials and their Characteristics:

1- *Escherichia coli:*

Escherichia coli (E. coli) is a stick non-sporulated Gram negative bacteria. It is an aerobic or facultative anaerobic species. It is grown at a temperature range between 35°C and 37 °C, but it can support until 44,5 °C. For this reason it is called thermo-tolerant coliform (31). The naturally occurring (wild-type) strain of *E. coli* doesn't require any growth factors. If given the appropriate elements and an energy source, *E. coli* can synthesize all 20 amino acids, all vitamins, all nucleotides, and all fatty acids that it uses during growth and metabolism(32).

8.1.7 Ampicillin Synthesis Steps

8.1.7.1 Preparation of the solution of ampicillin synthesis:(33)

- Weight 0.143 g commercial penicillin G (C=0.04g/ml) and dissolved it in 10 mL of potassium phosphate solution (pH=7 and C= 0.1 mol/L) in a volumetric flask
- Measure a volume of 7.5 mL from this mixture, using a graduated cylinder
- Add 0.8g of penicillin acylase (PGA)
- Agitating for 1h

- Add 7.5ml of the ester D-(-)-a-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

8.1.7.2 Ampicillin purification and harvesting:(34)

- Add few drops of H_2SO_4 to stop the enzymatic reaction
- Filtrate the mix using a funnel fitted with filter paper

  (Take 1ml of the filtrate for the bacterial sensibility test later)
- Add butyl acetate solvent (1Vsolvant/2Vfiltrate)
- Let rest for 2 minutes, then the organic phase is removed and the aqueous phase is discarded after decantation

  (Take 1ml from each phase to test the presence of ampicillin later)

  {We will carry out a qualitative, non-quantitative antibiogram, just to see the appearance or not of an inhibition zone following the application of: filtrate, the organic phase and the aqueous phase, stored in the ependorfs tubes (as already explained) on petri dishes containing Muller-Hinton medium. So, we are going to test the effectiveness of the antibiotic on the bacteria.}
- Add 2% phosphate buffer (V/V) to the organic solution
- Adjust the PH to 7.5 by adding NaOH

8.1.7.3 Crystallization: (35)

- Add 2% (W/V) NaHCO3 to the aqueous phase medium
- Cool them at 4°C for about 7 days
- Filtrate them to harvest ampicillin sodium salt

8.1.8 Ampicillin Quantification (Standard Protocol):

➢ <u>The one bacterial strain which can be used:</u> E. coli

➢ <u>Regeneration of Escherichia coli Bacteria</u>

- 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

➢ <u>Qualitative antibiogram, (non-quantitative antibiogram) using disc diffusion method</u>

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 4
- Deposit 3 disc in each quadrant with an empty quadrant used as control
- Add 20µl of each simple on a disc
- Reverse the plate and incubate it at at 37^{o} for 18 to 24h

➢ Preparation for commercial ampicillin quantification(36)

In order to carry out an antibiogram, from which the diameters of the zones of inhibition can be measured and the standard curve established

- Weigh 1g of commercial ampicillin
- Add 5 ml of potassium phosphate buffer to the ampicillin
- Filtrate the mix using a sterile funnel and filter paper
- Store the mix (C standard ampicillin=200mg/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**
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 muller 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
- Reverse the plate and incubate it at at 37° for 18 to 24h
- After the growth time, measured the zone of inhibition that had appeared using a ruler.
- Draw a graph showing the concentration of ampicillin as a function of the diameter in order to be able to quantify the produced ampicillin (diameter as a function of Log C).

بسم الله الرحمن الرحيم

AECENAR
Association for Economical and Technological Cooperation in the Euro-Asian and North-African Region

Lab Scale Production of Ampicillin With Ampicillin Quantification

Introduction:
Semi-synthetic penicillins antibiotics (SSPAs), one of the most important families of anti-infection drugs in the world market, are mainly producedby a two-step fashion. Ampicillin is one of the most widely used -lactam antibiotics in therapy as it is suitable for a wide spectrum of bacterial infections and has a good level of activity and tolerability. The qualitative analysis was done through β-lactamase test using penicillin or ampicillin resistant *Escherichia.coli* . Then quantitative analysis was performed by measuring the diameter of zones of inhibition of all the culture samples and comparing them with the standard curve drawn by measuring the diameter of zones of inhibition of standard dilutions of commercially available penicillin G or Ampicillin

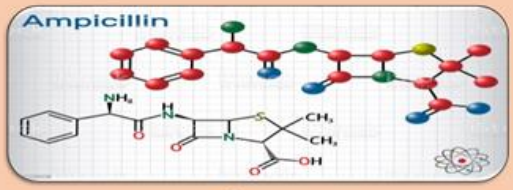
Ampicillin production:

To produce semi-synthetic β-lactam(Ampicillin), there are two proposed methods: One put-one step synthesis(1P1S) and one put two steps synthesis(1P2S) while the second has showed a most overall yield then the first.

1P2S:

1- Ampicillin synthesis:
The reaction was carried out in a round bottom flask on magnetic stir plate at T° 25° C under constant stirring.

- First we try to prepare 2 solution the first is NaOH (1M) and the second is H2SO4 (1M) which are important to adjust the PH of the reaction
- In order to add penG , We add 7.5 ml of 40 mM of powdered penG by using a phosphate buffer (100mM: PH 7)
- We add 0.8 g of penicillin G acylase (124 UpenG/gram of carrier) or 99.2 UPenG/gram of carrier
- We let the reaction to start with stirring and we wait about 60 minute before the ester will be added
- After 60 minute we add 7.5 ml of 120 mM D-PGMEH(D- Phenylglycine methyl ester hydrochloride)
- Then we add 0.24 g of penicillin G acylase (or 30 Upen G/ gram of carrier)
- We adjust the PH for about 6.4-7.0 by adding NaOH
- We let the rection to continue with stirring
- The second step need about 1290 min (22.5 h) to be achieved. So the total time needed by the reaction is 1350 min



2- Ampicillin purification and harvesting:

- After the reaction will be finished, H2SO4 is added in order to stop the enzymatic reaction.
- Then a solvant is added (ethyl acetate)
- Sodium bicarbonate solution of 6mM is added to the medium in order to obtain a solid phase.
- The last step is to filter and dry the solution to obtain ampicillin powder as ampicillin sodium Her the produced ampicillin is ready to be tested and quantified.

Proposed protocol for ampicillin quantification

1-Preparation of the turbidity calibration 0.5 McFarland(1):

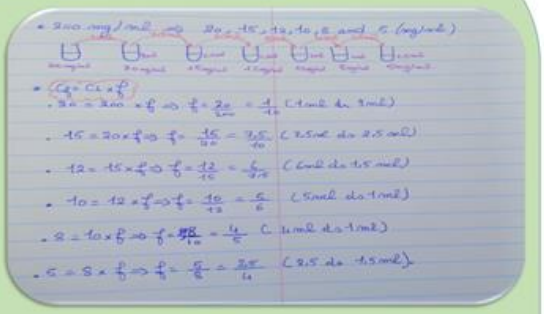
1. we add 0.5 mL of a 0.048 mol/L solution of $BaCl_2$ (1.175% w/v $BaCl_2$ $2H_2O$) to 99.5 mL of a 0.18 mol/L solution (0.36 N) of H_2SO_4 (1% v/v) and we shook vigorously

2.We check 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

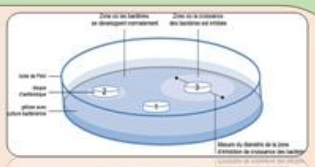
3.we distribute the suspension in tubes of the same size as those used to adjust the inoculum and then we seal the tube

4.once sealed, we store these tubes at room temperature and protect from light. Before use, we mix the tube vigorously using a Vortex (6 months' storage)

2- The one bacterial strain which can be used:
E.*coli*.



3- Quantification of the produced ampicillin using the disk diffusion method:



Preparation of the inoculum:
•We took 3 to 5 colonies of the isolated colonies with a loop, and we added them in 2ml sterile saline (NaCl 0.9%)
•We Vortex the saline tube to create a smooth suspension.
•We 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.
• Use this suspension within 15 minutes of preparation.
•We inoculate the surface of 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.
•We allow the plate to sit 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.

•**Preparation of the disks: we** dilute the standard ampicillin 6 times (Concentrations: 20;15;12;10;8;5 mg/ml) to obtain different concentrations.We distribute 16 disks in plates. On each disk, we add 20 µl of each ampicillin concentration obtained before, in order to have three disk with known concentration and one disk with unknown concentration per plate when we shall add our obtained produced ampicillin.
•Once all disks are in place, we replaced the lid, inverted the plate and placed it at 37°C for 18 to 24 hours.

Ghina Abdel Hamid – Hind Abd El Hamid
@MEGBI/AECENAR 2022

8.2 Materials and methods:

8.2.1 Production of ampicillin by the one-pot, two-step method (1P2S): (30)

This work has been done twice. The same experimental protocol was followed in Experiment 1 and Experiment 2, with the exception of a few modifications which will be clearly illustrated in the remainder of this part.

8.2.1.1 Bacterial strain used:(30)

A well-identified bacterial strain of *Escherichia coli* (ATCC 25922) obtained from the Doctoral School of Science and Technology in Tripoli, Northern Lebanon, was used in this work.

8.2.1.2 Products Used:(30)

Penicillin G, *Escherichia coli* penicillin G acylase immobilized on the Eupergit ® C commercial carrier and D-PGME were all purchased from **Carbsynth**. Also, the ethyl acetate solvent, obtained from the Chamber of Commerce, Industry, and Agriculture of Tripoli and North Lebanon, was used. Finally, the commercial ampicillin which is the ampicillin 1 g Panpharma powder for injection, obtained from Minapharm pharmacy, Tripoli, North Lebanon, was also used in this work.

8.2.1.3 Solutions Used:(30)

Potassium phosphate buffer solution, as well as pH, NaOH and H2SO4 adjustment solutions were used in this work. The protocols for the preparation of these solutions are detailed in the "Annexes" section of this report.

8.2.1.4 Ampicillin Synthesis:(30)

First, **a penicillin G solution (C=0.04 mol/L)** was prepared: A mass of 0.286 g (approximately 0.29 g) of Penicillin G (powder; M=356.4 g/mol^{-1}) was weighed using a digital balance, transferred into a beaker and then dissolved in 20 ml of the phosphate solution of potassium previously prepared (pH=7 and C=0.1 mol/L). From this solution, a volume of 15 mL was taken and transferred to a new beaker of 100 mL.

To this same beaker, the enzyme iPGA (powder) has been added: In fact, 124 units of penicillin G require 1 g of enzyme (where 1 Unit of Penicillin G acylase is defined as 1.0 mol of hydrolyzed penicillin G per minute). 198.4 units of penicillin G are required, so a mass of 1.6 g of iPGA was measured using the digital balance and added. The whole was well mixed, the magnetized bar was added to the beaker which was then placed on the magnetic stirrer to stir for 60 minutes at room temperature (from 22 to 25° C.). One hour later, 6-APA and PAA were obtained in the solution following the completion of the ^{first} reaction, iPGA-catalyzed hydrolysis of penicillin G .

Figure 31: Solution containing 6-APA and PAA obtained by iPGA-catalyzed hydrolysis of Penicillin G

Next, **an ester solution (C=0.12 mol/L), D-PGME (powder, M=201.65 g.mol^{-1})**, was prepared: A mass of 0.48 g of ester was measured using a digital balance and then transferred to a beaker, to which a volume of 20 mL of the previously prepared potassium phosphate solution (pH=7 and C=0.1 mol/L) was added to ensure the total dissolution of the ester. From the above solution, a volume of 15 ml was taken using a graduated test piece and transferred to the beaker containing the reaction mixture. Finally, the iPGA was added to the mixture again: In fact, 30 units of penicillin G require 1 g of iPGA, 14.4 units of penicillin G are needed, so a mass of 0.48 g of iPGA was measured using the digital balance and added to the beaker of the reaction.

The contents of the beaker were well mixed and the pH of the solution was measured using a digital pH meter. The latter showed a value of 6.50, the pH of the solution was then adjusted to the pH value=7 by adding a few drops of the strong base solution, NaOH, already prepared

Figure 32: Adjustment of the pH of the reaction mixture to the value 7

The beaker containing the reaction mixture was again placed on the magnetic stirrer to ensure stirring for 1270 min (approximately 22.5 h) until the completion of the 2nd reaction, which is the coupling of the 6-APA and the acyl (R)-PGME side chain catalyzed by the iPGA. The total time required to produce ampicillin is then 1350 min.

8.2.1.5 Filtration of the reaction mixture

After completion of the reaction, the beaker containing the reaction mixture was moved under the hood, and a few drops of the previously prepared H2SO4 strong acid solution were added to this beaker in order to block the reaction completely and prevent the degradation of ampicillin. Then, using a glass container and a funnel with a sterile filter paper, the filtration was carried out in order to remove the enzyme from the filtrate .

Figure 33: Filtration of the reaction mixture

After that, a few eppendorf tubes were filled with 1 mL of filtrate each using a pipette. This filtrate obtained will be called "**filtrate 1**" in the remainder of this work. The eppendorf tubes were subsequently used with the qualification and quantification tests in order to test the efficacy of the ampicillin produced in this **filtrate 1**, against *E. coli*, which is a strain sensitive to ampicillin. These tubes were kept cold until they were used.

8.2.1.6 Purification and harvesting of ampicillin produced

To the remainder of the filtrate, the ethyl acetate solvent was added with a different volume between the 1st and the 2nd experiment:

In experiment 1: The remaining filtrate volume was 22.4 mL. Ethyl acetate has

was added according to the ratio 14:2, thus: 14 mL of filtrate require 2 mL of solvent; therefore, for 22.4 mL of filtrate, a volume of 3.2 mL of this solvent was added to the container containing the filtrate.

In experiment 2: The remaining filtrate volume was 6 mL. Ethyl acetate was

added according to the ratio v:v, therefore 6 mL of filtrate require 6 mL of solvent. A volume of 6 ml of solvent was then added to the filtrate.

This step was carried out in order to make the ampicillin produced soluble in the ethyl acetate solvent in order to recover it subsequently in the organic phase only. Following the addition of this solvent, the mixture was divided into two phases: the aqueous phase and the organic phase. The mixture was then transferred to a glass tube in order to better visualize the separation .

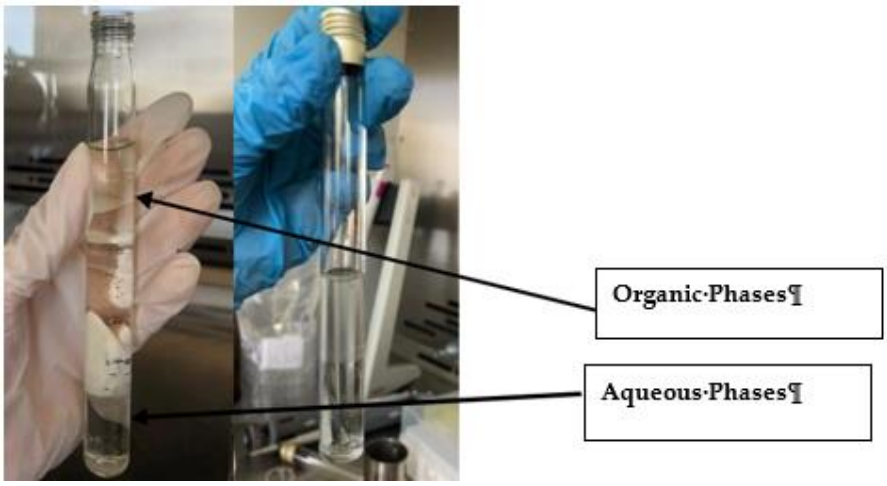


Figure 34: Separation of the reaction mixture into organic (top) and aqueous (bottom) phases from experiment 1 (left) and experiment 2 (right)

Then, using a pipette fitted with a pro-pipette, the organic phase was removed and transferred to a new tube. The aqueous phase, which should be eliminated, was also preserved and tested later for curiosity. Thus, from the aqueous and organic phases, eppendorf tubes were filled, each approximately 1 mL, using a graduated pipette equipped with a pro-pipette. These tubes were kept cold for later use in qualification and quantification tests to test the presence and efficacy of ampicillin in these phases.

The next step is to recover **pure ampicillin** from the remaining organic phase, while adding sodium bicarbonate to this phase. Following its addition, the crystallization process will take place finally producing pure ampicillin powder form (solid phase). **This step was carried out only in experiment 1.** Indeed, sodium bicarbonate was added with a ratio of 10:1.44; thus, 10 mL of the organic phase require 1.44 g of sodium bicarbonate (powder), therefore for 1.4 mL of the organic phase, a mass of 0.2016 g of sodium bicarbonate was added, and then the tube was preserved cold for 24 h until the crystallization process is complete. Following its completion, a white deposit was noticed at the bottom of the tube, thus the last step to be carried out **experiment 1** consists in filtering and drying the residue of the glass tube containing the organic phase, in order to obtain ampicillin powder.

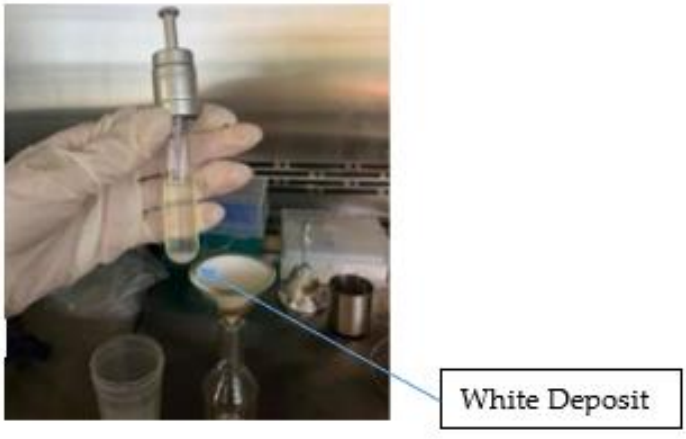


Figure 34: Filtration of the contents of the tube containing the organic phase following the crystallization process

Figure 35: Harvesting the Ampicillin Powder Produced

In order to preserve the efficacy of the antibiotic, the ampicillin powder was dissolved in 3 mL of the previously prepared potassium phosphate buffer solution (pH=7; C=0.1M). The last step of this **experiment 1** consists in carrying out the filtration, the filtrate obtained has been named **filtrate 2**. This filtrate was then transferred into 2 eppendorf tubes of volume 1 mL each, which has been kept cold, and subsequently used to quantify ampicillin in this filtrate.

Figure 36: Eppendorf tubes containing filtrate 2 from the 1st experiment

All the above-mentioned steps following that of the addition of the ethyl acetate solvent, therefore starting from the sodium bicarbonate addition step, were not carried out in the 2nd experiment.

8.2.2 Qualification testing of ampicillin produced:

A. Qualitative antibiogram

This test was carried out **only for experiment 1**. The aim is to test the efficacy of ampicillin produced against *E.coli*, which is a strain susceptible to ampicillin. Thus, a qualitative antibiogram was carried out, just to see the appearance or not of an inhibition zone bacterial growth following the application of: **filtrate 1**, the organic phase and the aqueous phase, which have been stored cold in eppendorf tubes, on petri dishes containing the Mueller-Hinton medium (prepared in advance).

Figure 37: Well-identified bacterial strain of E. coli (ATCC

For this purpose, a well-identified *E.coli* bacterial strain (ATCC) was used (Figure 11).

Using a sterile loop, a bacterial colony was taken from this strain and grown on a petri dish containing the standard agar medium prepared in advance. The culture was ready 24 h after incubation and thus bacterial regeneration was carried out. From this bacterial culture of E. coli, an isolated colony was collected using a sterile loop, the loop was then soaked in a sterile saline solution, NaCl 0.9% (hence 9 g of NaCl in 100 mL of distilled water), and the contents of the tube were well mixed. The turbidity of this tube was compared with that of the McFarland Standard tube (containing a standard 0.5 MF BaCl2 solution).

The same turbidity should be obtained by adding more colonies if the suspension is too light or by diluting with a sterile saline solution, 0.9% NaCl (already prepared in a separate tube), if the suspension is too heavy, until this condition is met.

Figure 38: Comparison of turbidity between the bacterial suspension tube (right) and the standard McFarland tube (left).

A sterile swab was soaked in the bacterial suspension tube. Press firmly against the inner wall of the tube just above the liquid level and turn the swab to remove excess liquids. Then the

spreading was done three times, turning the can at about 60°C after each application to obtain an equal distribution of the inoculum over the entire surface of the Mueller-Hinton agar. Finally, swabs were performed all around the edge of the agar surface. These steps were carried out for 3 petri dishes. The cans were inverted and each divided into 2 parts: a box for the comparison between the **filtrate 1** and the organic phase (1st box); a second for the comparison between the **filtrate 1** and the aqueous phase (2th box) and finally a control box. After that, the disks were deposited using a sterile clamp. In addition, a 20 L volume of the eppendorf tube containing the filtrate 1 was deposited on the disk at this filtrate of the 1st and 2th cans, respectively, by means of a pipette; a 20 L volume of the eppendorf tube containing the organic phase was taken and then deposited on the disk corresponding to this phase on the 1st cans and finally, a 20 L volume of the eppendorf tube containing the aqueous phase was taken and then deposited on the disk corresponding to this phase on the 2nd box (Fig.13). The 3 petri dishes were then placed in the incubator for 18 to 24 h at 37°C.

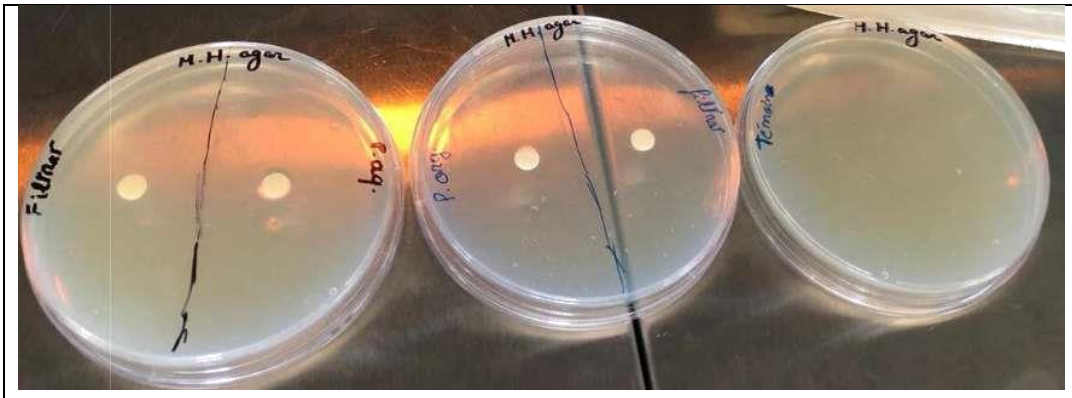


Figure 39: Qualitative antibiogram for filtrate 1, aqueous phase and organic phase of experiment 1 on petri dishes containing Mueller Hinton agar

B. <u>Sensitivity test</u>

This test was carried out **only for experiment 2**. The aim is also to test the presence and efficacy of ampicillin produced against *E.coli*, an ampicillin-sensitive strain. The standard agar medium was prepared in advance and poured into 3 petri dishes. On the first box, the **filtrate 1** (which is the only filtrate obtained in this experiment) stored in the eppendorf tube was poured. You have to move the box to cover the whole middle. After 2 to 3 minutes, the excess filtrate was discarded from the surface, then, using a sterile loop, a colony of *E. coli* was taken from a bacterial culture already prepared, and was striated on the surface of the medium. Spreading was carried out from the top of the box downwards, covering the entire surface. In the same way, the two other cans, one corresponding to the organic phase and the other to the aqueous phase, were prepared. The cans were then placed in the incubator for 18 to 24 h at 37°C.

8.2.3 Quantification Tests for Ampicillin Produced

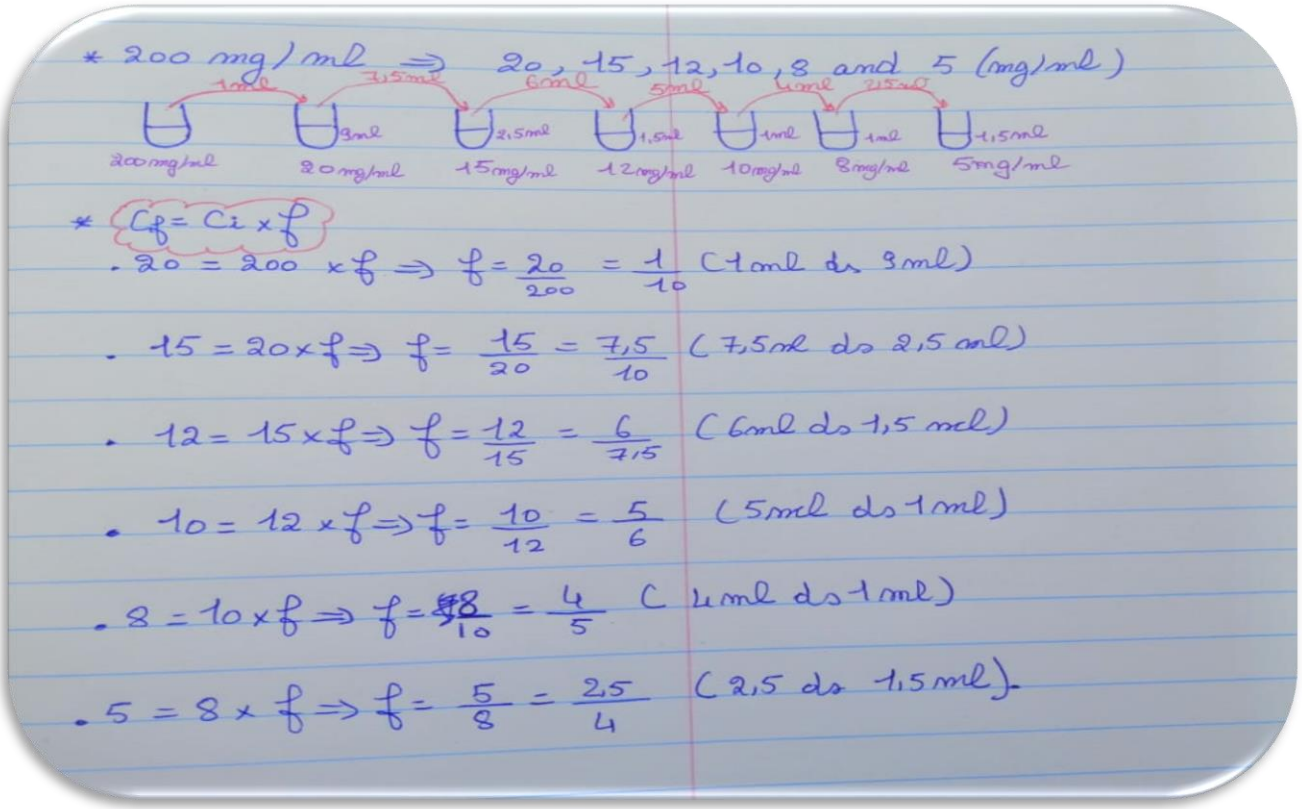
The quantification of the ampicillin produced was carried out using the disk diffusion method, the principle of which is as follows: Cellulose disks, impregnated with a given quantity of an antibiotic, are placed on an agar, previously inoculated by the strain to be tested (in our case, an *E.coli* strain). The antibiotic diffuses from the disk into the agar, with a

decreasing concentration gradient, from the disk to the periphery. After incubation for 18 hours at 24 hours at 37° C., a measurement of the zones of inhibition of bacterial growth is carried out for each of the disks. The larger the diameter, the more sensitive the bacterium is to the antibiotic tested.

8.2.3.1 Preparation of a standard ampicillin solution and carrying out a series of dilutions:

To quantify our produced ampicillin, first, we need a standard ampicillin solution that will be used as standard to draw a standard curve.

1) **Preparation of the disks:**
   - First we need to prepare ampicillin stock solution, for this reason we dissolve 1 g commercial ampicillin sodium in 5 ml PBS (PH=7/ 0.1M) to obtain a 200 mg/ml standard ampicillin solution
   - we dilute the standard ampicillin 6 times (Concentrations: 20;15;12;10;8;5 mg/ml) to obtain different concentrations.



2) **Preparation of the inoculum:**
   - We took 3 to 5 colonies of the isolated colonies with a loop, and we added them in 2ml sterile saline (NaCl 0.9%)
   - We Vortex the saline tube to create a smooth suspension.
   - We 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.
   - Use this suspension within 15 minutes of preparation.

- We inoculate the surface of 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.
- We allow the plate to sit 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.
- We distribute 16 disks in plates. On each disk, we add 20 µl of each ampicillin concentration obtained before, in order to have three disks with known concentration and one disk with unknown concentration per plate when we shall add our obtained produced ampicillin.
- Once all disks are in place, we replaced the lid, inverted the plate and placed it at 37°C for 18 to 24 hours.

The test for quantification of the ampicillin produced was carried out first for the **filtrate 2** of **the experiment 1**; and then for the **filtrate 1** and the organic and aqueous phases. Whereas for **experiment 2**, the quantification of the ampicillin produced was carried out for the **filtrate 1** and the organic and aqueous phases. Thus:

Quantification of filtrate 2 from experiment 1: The dishes were divided into 4 regions:

- 3 for known concentrations

and

- 1 for unknown ampicillin concentration in **filtrate 2.**

The 1st dishes and its copy were then divided for the concentrations: 20, 15, 12 mg/mL and the unknown concentration of ampicillin in **filtrate 2**. The second box and its copy for concentrations 10, 8, 5 mg/mL as well as the unknown concentration of ampicillin in **filtrate 2**.

Quantification of filtrate 1 and the organic and aqueous phases of experiment 1 and experiment 2:

One dishes was divided into 4 regions for concentrations 20, 15, 12 mg/mL and the unknown concentration of ampicillin in **filtrate 1** (same for its copy can); and the another was divided into 5 regions for concentrations 10, 8, 5 mg/mL and the unknown concentration of ampicillin in the aqueous phase and in the organic phase.

8.2.4 Experiment 3: First Trial of Ampicillin crystallization:

The same steps are applicable to produce ampicillin

Here two method of the crystallization can be applicable. The first one is mentioned below:

8.2.4.1 Ampicillin purification and harvesting:

- Add few drops of H_2SO_4 to stop the enzymatic reaction
- Filtrate the mix using a funnel fitted with filter paper

  (Take 1ml of the filtrate for the bacterial sensibility test later)
- Add butyl acetate solvent (1Vsolvant/2Vfiltrate)

- Let rest for 2 minutes, then the organic phase is removed and the aqueous phase is discarded after decantation

  (Take 1ml from each phase to test the presence of ampicillin later)

  {We will carry out a qualitative, non-quantitative antibiogram, just to see the appearance or not of an inhibition zone following the application of: filtrate, the organic phase and the aqueous phase, stored in the Eppendorf tubes (as already explained) on petri dishes containing Muller-Hinton medium. So, we are going to test the effectiveness of the antibiotic on the bacteria.}

- Add 2% phosphate buffer (V/V) to the organic solution
- Adjust the PH to 7.5 by adding NaOH

Crystallization

- Add 2% (W/V) NaHCO3 to the aqueous phase medium
- Cool them at 4oC for about 7 days
- Filtrate them to harvest ampicillin sodium salt
- Then after 10 days we observed the formation of thin crystals which must be tested based on the sensitivity of E.coli against ampicillin.

8.2.5 Experiment 4: Second trial of ampicillin crystallization

- The same steps of production mentioned above are followed
- Then, after 22.5 h of agitation of the mix, the reaction is stopped by adding few drops of H2SO4 and the PH is adjusted to 2, here the ampicillin is presented ionized in the solution.
- The mix is then filtrated using filter paper, 10 mL of butyl acetate is added as solvent to 20 mL filtrate, the organic phase is taken after decantation and the PH is adjusted to 4 by adding NaOH then (2% W/V) NaHCO3 is added. Ampicillin crystals are formed.
- The mix is cooled down at 40 and then filtrated so ampicillin sodium salt are obtained.
- After crystallisation the crystals are dissloved in 2 ml phosphate buffer , filtrated and then quantified by using microbiological essay as mentioned previously.

8.3 Results and discussion:

8.3.1 Results of the experiment 1

8.3.1.1 Qualification Test for Produced Ampicillin

The result of the qualitative antibiogram shows the appearance of a zone of inhibition of bacterial growth with the **filtrate 1**, the aqueous phase and the organic phase. This confirms the presence of ampicillin produced in these 3 phases, as well as its efficacy since it inhibited the bacterial growth of *E. coli* contrary to the result obtained in the control box where there was normal bacterial growth (FIG. 14).

Figure 40: Result of the qualitative antibiogramme of filtrate 1, the aqueous phase and the organic phase of experiment 1

8.3.1.2 Quantification Tests for Ampicillin Produced:

A. <u>Quantification of ampicillin produced in filtrate 2:</u>

Firstly, the quantification test for the ampicillin produced was carried out for filtrate 2. The antibiogram showed the appearance of bacterial growth inhibition zones with all concentrations (20; 15; 12; 10; 8 and 5 mg/ml) obtained from the dilution of the standard ampicillin solution (C=200 mg/ml), however no inhibition zone appeared with **filtrate 2** .

On the basis of the above-mentioned results, the 2nd experiment was limited to carrying out the qualification and quantification tests just for the **1 st filtrate**, obtained following the production and filtration of the reaction mixture, and for the aqueous and organic phases obtained following the first step of the purification which consists in adding the ethyl acetate solvent.

Thus, for the 2nd experiment, qualification and quantification tests were carried out after each stage directly, without going through the process of obtaining **the ampicillin produced pure** from the organic phase which requires adding the sodium bicarbonate to this phase, carrying out the filtration and recovering the ampicillin powder, which in turn must be dissolved in the buffer solution in order to preserve its effectiveness. Also, the 2nd experiment was carried out by adjusting a parameter of the purification of the ampicillin produced, which is the

increase in the volume of solvent added, with the aim of optimizing the purification and recovering the ampicillin produced only in the organic phase.

B. Quantification of the ampicillin produced in filtrate 1 and the organic and aqueous phases:

The antibiogram showed the appearance of bacterial growth inhibition zones at all concentrations (20; 15; 12; 10; 8 and 5 mg/ml) obtained from the dilution of standard ampicillin solution, as well as the appearance of growth inhibition zones bacterial cells which are reduced, with **filtrate 1**, and the aqueous and organic phases. The same result was obtained for each copy box.

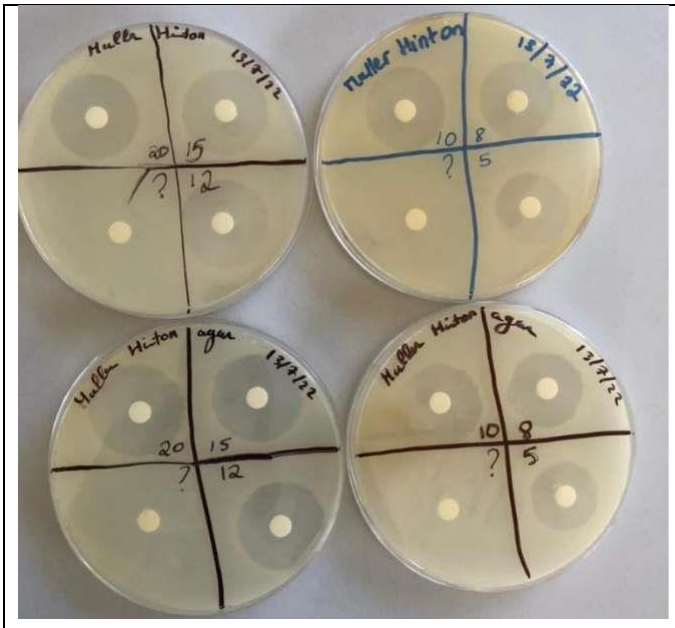


Figure 41: Result of the Quantitative Antibiogram of the filtrate 2 of Experiment 1

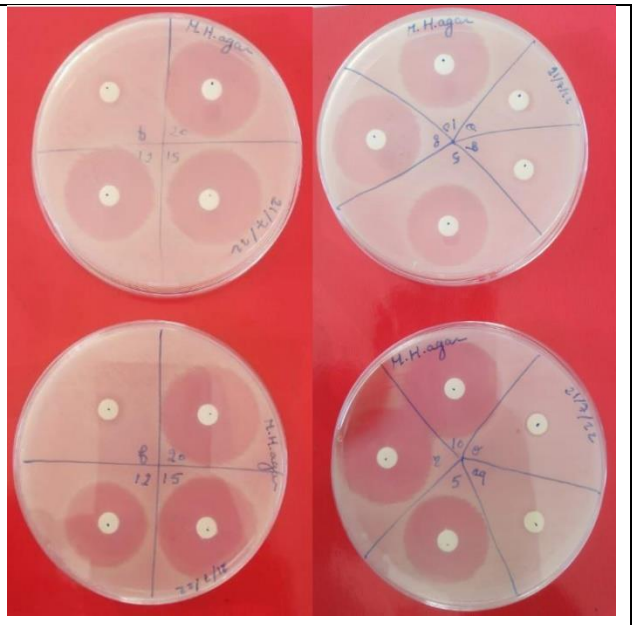


Figure 42: Result of the quantitative antibiogram of filtrate 1 and of the aqueous and organic phases of experiment 1

Subsequently, the diameters of the inhibition zones relative to the different concentrations of standard dilute ampicillin, as well as those of filtrate 1 and of the organic and aqueous phases were measured by means of a ruler in each box and its copy, and then the mean was calculated (Table 4):

Table 4: Measurements of diameters of bacterial growth inhibition zones for different concentrations of standard dilute ampicillin and filtrate 1, aqueous phase and organic phase of experiment 1

Concentration (mg/mL)	20	15	12	10	8	5	Filtrat 1	Phase organique	Phase aqueuse
Log C	1.3	1.18	1.08	1	0.9	0.69	?	?	?
Diamètre (cm)	$\frac{3.1+3}{2}$ = 3.05	$\frac{2.9+3.1}{2}$ = 3	$\frac{2.8+2.5}{2}$ = 2.65	$\frac{2.9+2..8}{2}$ = 2.85	$\frac{2.8+2.8}{2}$ = 2.8	$\frac{2.5+2.6}{2}$ = 2.55	$\frac{1+1}{2}$ = 1	$\frac{0.8+0.8}{2}$ = 0.8	$\frac{0.8+0.8}{2}$ = 0.8

Next, the graph showing the concentration of ampicillin as a function of the diameter of the inhibition zone (Log C as a function of diameter) was drawn in order to be able to quantify the ampicillin produced in filtrate 1, the organic phase and the aqueous phase (FIG. 43):

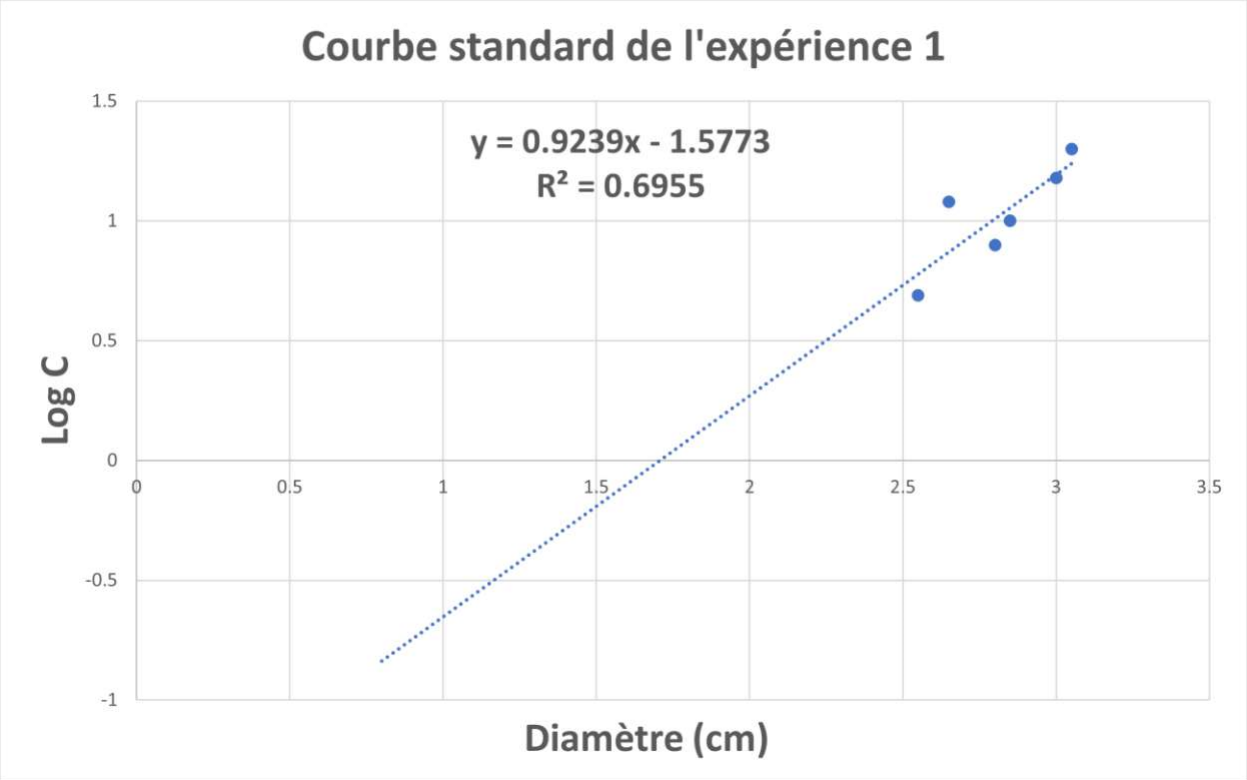


Figure 43: Graph showing the variation in ampicillin concentration (Log C) as a function of the diameter of the inhibition zone, corresponding to experiment 1

From the following equation the concentration of the ampicillin produced in **filtrate 1**, the aqueous phase and the organic phase is determined

$$y=0.9239x-1.5773$$

For **filtrate 1**:

$$Log\ C=0.9239\ x\ 1-1.5773=-0.6534\ ;\ C=0.222\ mg/ml$$

For organic and aqueous phases having the same diameter of their inhibition zones:

$$Log\ C=0.9239\ x\ 0.8-1.5773=-0.83818\ ;\ C=0.145\ mg/ml$$

8.3.2 Results of the experiment 2

8.3.2.1 Qualification Test for Produced Ampicillin

The result of the bacterial sensitivity test carried out for **filtrate 1** (the only filtrate obtained in this experiment) and the aqueous and organic phases shows an inhibition of the bacterial growth thus confirming again the presence of ampicillin in filtrate 1 (FIG. 44) and in the other two phases (FIG. 45), as well as its effectiveness.

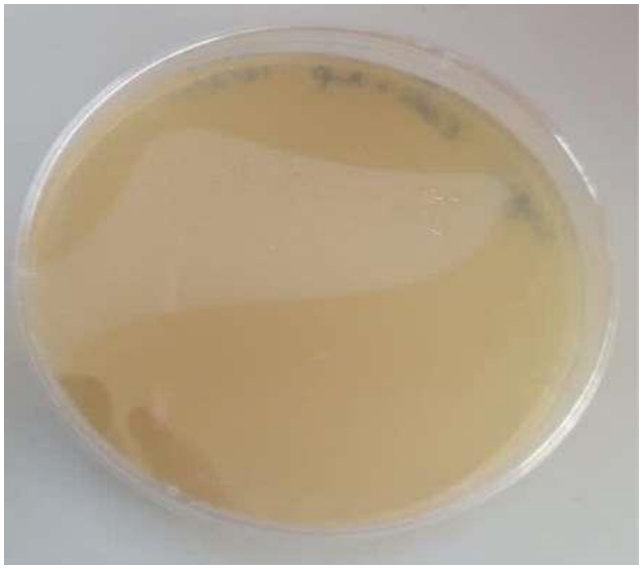


Figure 44: Bacterial susceptibility test to ampicillin contained in the filtrate 1 of experiment 2

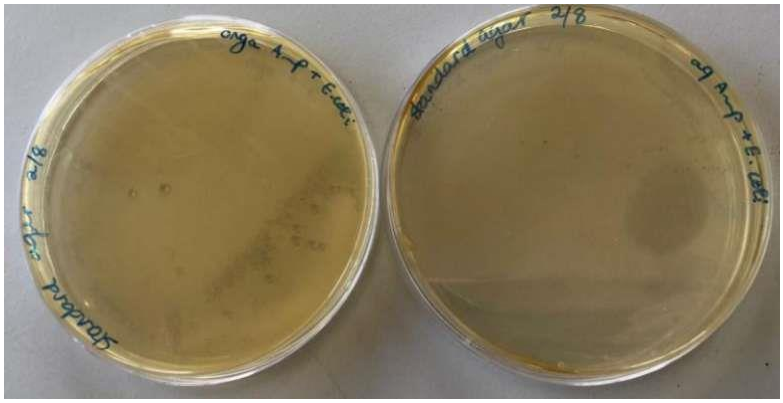


Figure 45: Bacterial susceptibility test to ampicillin contained in the organic and aqueous phase of experiment 2

8.3.2.2 Quantification Test for Ampicillin Produced

Also in this experiment, the antibiogram showed the appearance of the bacterial growth inhibition zones with all the concentrations (20; 15; 12; 10; 8 and 5 mg/ml) obtained from the dilution of the standard ampicillin solution, as well as the appearance of reduced bacterial growth inhibition zones with filtrate 1 and the aqueous and organic phases. The same result was obtained for each copy box.

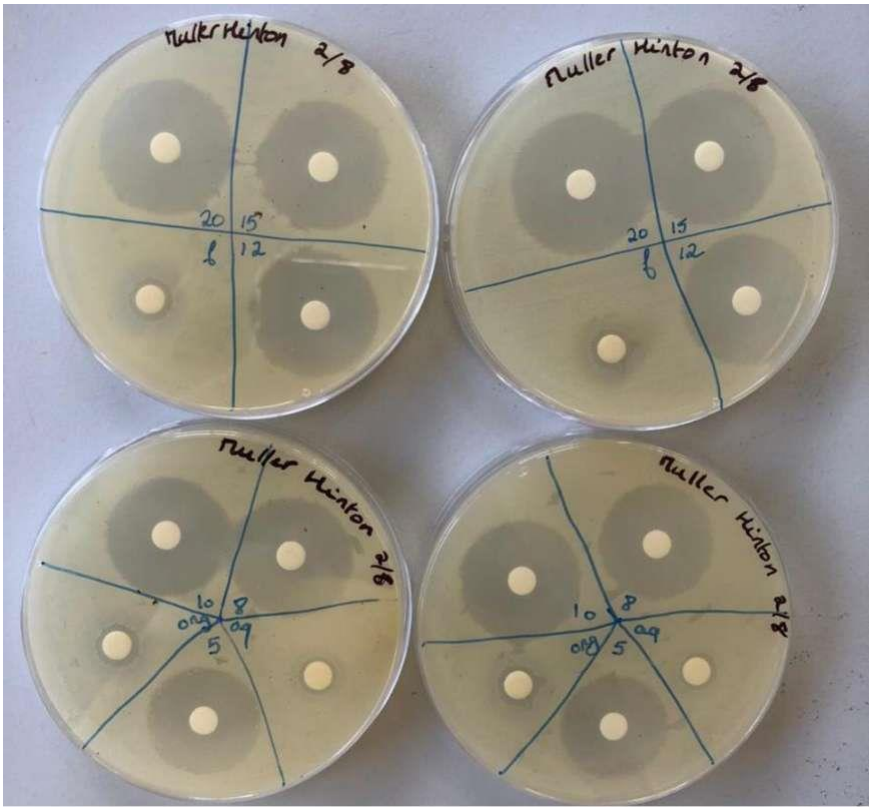


Figure 46: Quantification results

Then, in order to quantify the ampicillin produced in each of these phases, the diameters of the inhibition zones relating to the different concentrations of standard diluted ampicillin as well as those of **filtrate 1** and of the organic and aqueous phases were measured by means of a ruler in each box and its copy, and then the mean was calculated (Table 5):

Table 5: Measurements of diameters of bacterial growth inhibition zones for different concentrations of standard dilute ampicillin and filtrate 1, aqueous phase and organic phases of experiment 2

Concentration (mg/mL)	20	15	12	10	8	5	Filtrat 1	Phase organique	Phase aqueuse
Log C	1.3	1.18	1.08	1	0.9	0.69	-	?	?
Diamètre (cm)	$\frac{3+3}{2} = 3$	$\frac{2.9+2.85}{2} = 2.875$ ($\approx$2.9)	$\frac{2.8+2.85}{2} = 2.825$ ($\approx$ 2.8)	$\frac{2.7+2.65}{2} = 2.675$ ($\approx$ 2.7)	$\frac{2.65+2.6}{2} = 2.625$ ($\approx$ 2.6)	$\frac{2.5+2.4}{2} = 2.45$	$\frac{1.3+1.2}{2} = 1.25$	$\frac{1+1}{2} = 1$	$\frac{0.9+0.9}{2} = 0.9$

Next, the graph showing the ampicillin concentration as a function of the diameter of the inhibition zone (Log C as a function of the diameter) was drawn :

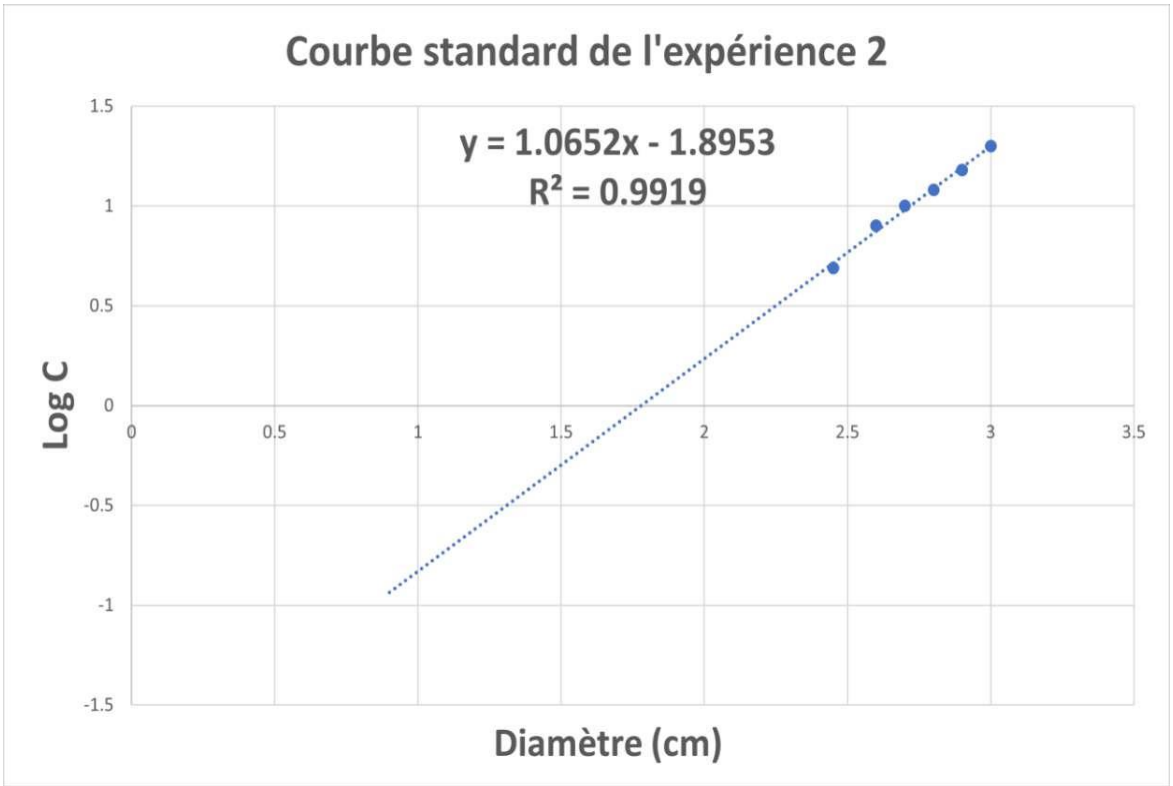


Figure 47: Graph showing the variation in ampicillin concentration (Log C) as a function of the diameter of the inhibition zone, corresponding to experiment 2

The concentration of ampicillin produced in filtrate 1, the aqueous phase and the organic phase is determined from the following equation:

$$y=1.0652x-1.8953$$

- For **filtrate 1**:

$$Log\ C=1.0652\ x\ 1.25-1.8953=-\ 0.5638;\ C=0.27\ mg/ml$$

- For the organic phase:

$Log\ C=1.0652\ x\ 1-1.8953=-\ 0.8301$ Then $C=0.15\ mg/ml$

- For the aqueous phase:

$Log\ C=1.0652\ x\ 0.9-1.8953=-\ 0.93662$ Then $C=0.12\ mg/ml$

8.3.3 Results of the Experiment 3: First Trial of Ampicillin crystallization:

Figure 48: Thin Ampicillin crystals

8.3.4 Results of experiment 4: Second trial of ampicillin crystallization

Figure 49: Ampicillin crystals obtained from the second trial

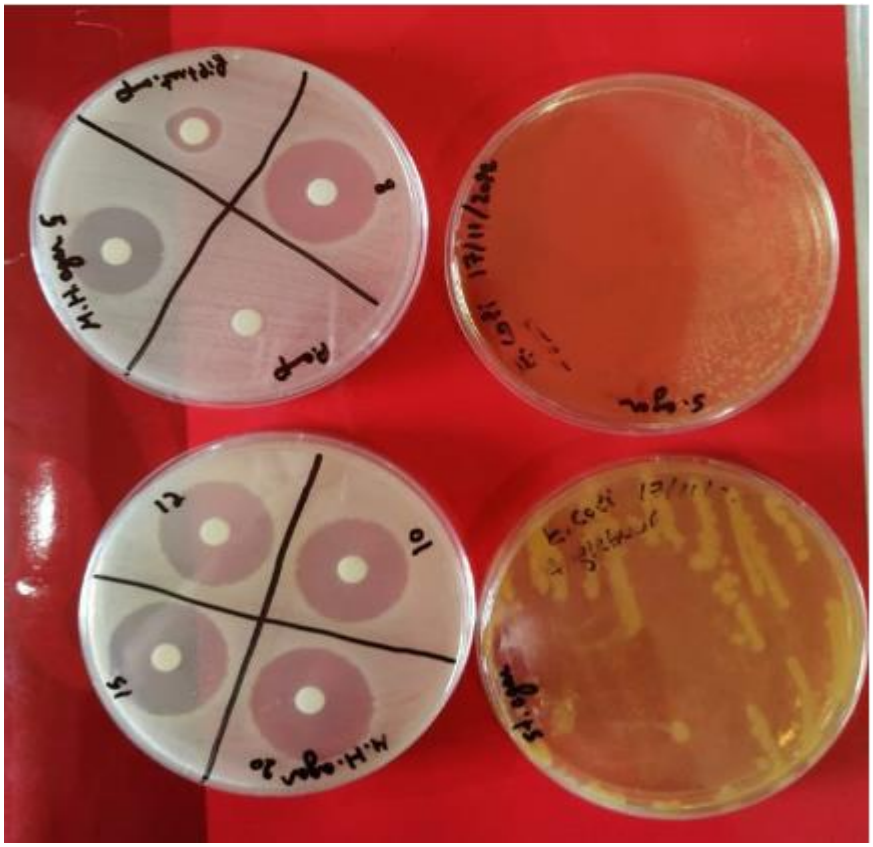


Figure 50: Microbiological essay results

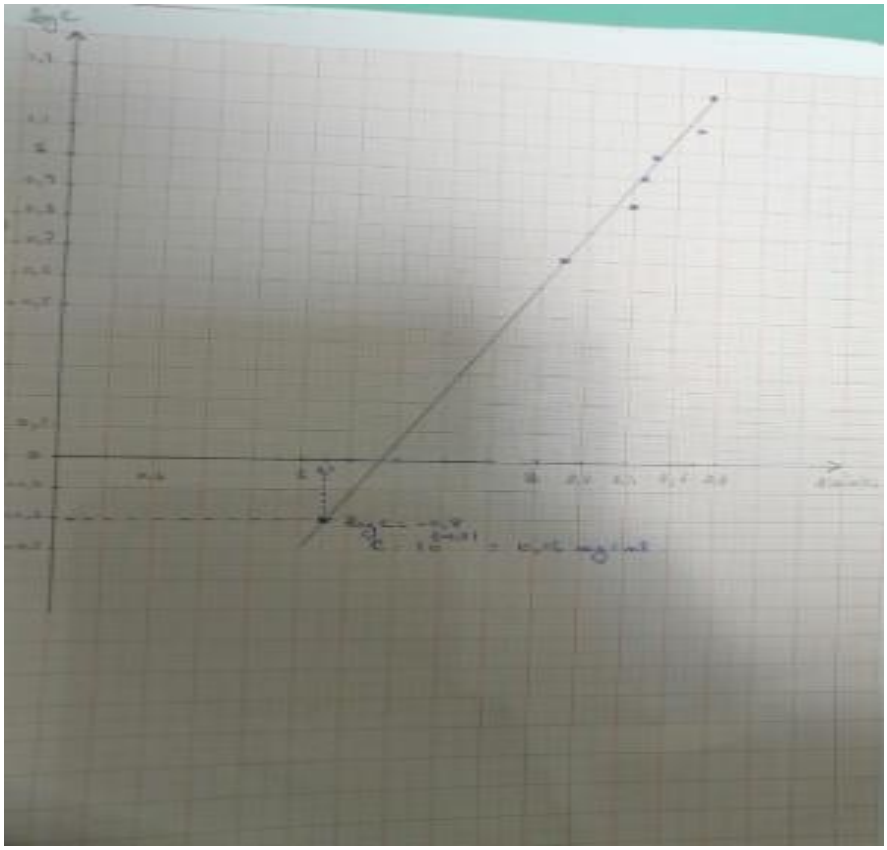


Figure 51: Standard curve to determine the concentration of ampicillin crystal after crystallizations

Based on the standard curve, the measure of diameter of the produced ampicillin equal to1.1 cm then graphically log C =-0.8

So

$C=10^{\log C}= 10^{(-0.8)}=0.16$ mg/ml

8.3.5 Discussion

Ampicillin is one of the most widely used antibiotics in therapy because it is suitable for a wide spectrum of bacterial infections and a good level of activity and tolerance. To date, numerous developments have been acquired in the enzymatic synthesis of ampicillin and the kinetically controlled strategy has proved effective. The aim of this work is to carry out cascade conversion with two biocatalytic reactions: the hydrolysis of penicillin G, and the kinetically controlled enzymatic synthesis catalyzed by penicillin G acylase immobilized from the (R)-phenylglycine methyl ester side chain and 6-aminopenicillanic acid containing the core of the lactam, in order to synthesize ampicillin in a fully aqueous medium according to the two-step, one-pot synthesis method. The aim was then to quantify the ampicillin produced.

With regard to our first production test for ampicillin **(experiment 1)**, the qualification test for the ampicillin produced confirmed the presence of this antibiotic in **filtrate 1**, the aqueous phase and the organic phase. However, the appearance of a zone of inhibition of bacterial growth with the aqueous phase was a surprising and unexpected result. Indeed, this result suggests the possibility of having a problem in the purification, since the addition of ethyl acetate was carried out in order to make the ampicillin produced soluble in this solvent, which should then extract the ampicillin from the aqueous phase and transfer it to the organic phase, thus making it possible to **recover this ampicillin only in the organic phase**. Thus, a loss of the amount of ampicillin produced in the aqueous phase is predicted. On the other hand, since the ampicillin produced was present in the **filtrate 1** and the aqueous and organic phases, its presence in the filtrate 2 which must contain the purified produced ampicillin was predicted, so it went directly to the stage of its quantification, without carrying out a qualification test for this filtrate 2. In this context, the antibiogram carried out showed the absence of the ampicillin produced in **filtrate 2.** In fact, this result was also unexpected since **filtrate 2** is supposed to contain **the pure produced ampicillin**, unlike **filtrate 1** which contains the unpurified produced ampicillin, and it is for this reason that attempts were made to quantify the produced ampicillin contained in **filtrate 2** first. However, this result could be explained as follows:

In the light of the result of the qualitative antibiogram of this first experiment, a small diameter of the inhibition zone corresponding to the **filtrate 1 is** noted, which already reflects a low concentration of ampicillin produced in the reaction. On the other hand, since the volume of the reaction mixture was so small, this suggests that the amount of ampicillin produced may be small. Furthermore, the problem in the first stage of purification, that of adding the ethyl

acetate solvent, seems to cause, from our point of view, the distribution of the ampicillin produced between the aqueous phase and the organic phase, thus further reducing the amount of ampicillin that should be obtained in the organic phase, which seems to lead to a reduction in the concentration of ampicillin produced in this phase, compared with a normal case where there is no purification problem. This seems to be logical, given the diameter of the inhibition zone corresponding to the organic phase which is reduced, or even smaller than that of the **filtrate 1**. In recalling the process of obtaining the **2nd filtrate**, which is a rather lengthy process: given that it was from the organic phase that the second filtrate was obtained, following the crystallization process with the addition of sodium bicarbonate to this phase, filtration, recovery of the pure ampicillin in powder form, dissolution of this powder in the potassium phosphate buffer solution and finally filtration again which gave this **filtrate 2,** it is noted that there has been a **significant dilution,** in our opinion, of the concentration of the pure ampicillin produced from the organic phase, and this dilution has taken place more precisely with the stage of dissolution of the pure powder obtained with the potassium phosphate buffer solution, which could be the origin of the obtaining a concentration of pure ampicillin in **filtrate 2 which is even** lower than the concentration of ampicillin in the organic phase. It seems to us that the concentration of pure ampicillin contained in filtrate 2 was then very low, so that it became lower than the minimum concentration of inhibition of bacterial growth, which could explain the absence of the appearance of zone of inhibition of bacterial growth with **filtrate 2**.

Then, after obtaining a negative result with the test for quantification of the ampicillin produced in filtrate 2, and since the qualification test previously carried out for the filtrate 1 and the aqueous and organic phases confirmed the presence of the ampicillin produced in these phases, it was then sought to quantify the ampicillin produced in each of these phases. As a result, the concentration of the produced ampicillin obtained in the **filtrate 1** is low, and it is close to the sum of those obtained in the organic and aqueous phases. Our hypothesis, which has already been put forward as to the existence of a problem in the purification, which leads to the distribution of the produced ampicillin between the organic phase and the aqueous phase, thus seems to be logical, while recalling that the aqueous phase must not contain the ampicillin produced under normal conditions of the purification. Indeed, since the ethyl acetate solvent used was unable to extract the ampicillin from the aqueous phase to the organic phase, it was then necessary to carry out the experiment a second time by trying to optimize the 1st stage of the purification, while increasing the volume of the solvent added to the **filtrate 1**.

When our 2nd experiment was carried out, the qualification test of filtrate 1 and of the aqueous and organic phases showed the presence of ampicillin in these phases. Thus, despite the adjustment of the volume of the solvent added, the problem in the purification of the ampicillin produced has not been solved since ampicillin is still obtained in the aqueous

phase. Then, the quantitative test was carried out, the concentration of the ampicillin produced obtained in the **filtrate 1** is also low, and almost equal to the sum of those obtained in the organic and aqueous phases, a result similar to that obtained with the 1st experiment. Next, it should be noted that the adjustment of a parameter in the purification of the ampicillin produced, which consists in increasing the volume of the ethyl acetate solvent added to the filtrate 1 of this 2th experiment could not correct the problem of the purification since ampicillin is always obtained in the aqueous phase, the ampicillin produced seems to be always distributed between the organic and aqueous phase. However, the concentration of the ampicillin recovered in the organic phase was this time greater than that obtained in the aqueous phase, which is more logical. Also, the second experiment showed a better result with respect to the standard curve with R^2=0.9919 compared to that of the first experiment where R^2=0.6955. It can be concluded that the optimization which was carried out by adding a volume of solvent according to the ratio v:v in the 1st stage of the purification improved the results, but these results do not deny the assumption that the ethyl acetate solvent is unable to completely extract ampicillin from the aqueous phase. In this context, the production of ampicillin and the quantification of the ampicillin produced have been completed, but the purification and harvesting of this antibiotic have not been successful.

In 2003, Wei, Dong-Zhi and Liu Yang in their study on the effect of ethylene glycol on ampicillin synthesis using penicillin G acylase showed that the yield of ampicillin synthesis can be significantly improved in the presence of ethylene glycol. Ethylene glycol was found to increase ampicillin yield by 39-50%. Indeed, the yield of ampicillin synthesis depends on the three different kinetic processes catalyzed by penicillin G acylase: ampicillin synthesis and enzymatic hydrolysis reactions. Therefore, the prevention of hydrolysis of the acyl donor substrate (D-PGME) and the product (ampicillin) is desirable for increasing the yield of ampicillin synthesis. With this strategy, organic solvents, including ethylene glycol, are often added to the aqueous medium to suppress enzyme hydrolysis reactions.

In 2009, a one-pot, two-step enzymatic synthesis of ampicillin in aqueous phosphate buffer using ethylene glycol as solvent was demonstrated for the first time by Du, Li-Li, et al. The ethylene glycol solvent was added in the v:v ratio and the maximum yield was 57.3%. Thus, it has also been demonstrated that the use of ethylene glycol has improved the yield of the synthesis. We have not used this solvent, it is then desirable to carry out the synthesis of ampicillin subsequently following the cascade conversion, in one pot, two steps using this solvent.

In 2010, Blum, Janna K., et al. conducted a study on the synthesis of ampicillin using a two-enzyme cascade, using the enzyme 15-amino ester hydrolase (AEH) and penicillin G acylase. The one-pot, two-step synthesis system gave an optimal ampicillin yield of 46%. Maximum conversions were obtained in one to two hours, significantly reducing the reaction times previously observed in systems using iPGA and ethylene glycol. It has been shown that the two-enzyme system with the iPG **and** AEH outperformed systems that used only the iPGA,

reporting the obvious benefit of using the AEH enzyme. In fact, AEHs are unique in their specificity to the phenyl-amine groups on the acyl moiety so that they cannot catalyze the hydrolysis of penicillin G to give 6-APA and are not inhibited by PAA, hence their advantage in this cascade.

In short, the cascade conversion was carried out with two biocatalytic reactions in a fully aqueous medium to synthesize ampicillin, following the two-step, one-pot synthesis method catalyzed by the iPGA. In light of these data, our work could be improved with the use of the ethylene glycol co-solvent or by performing the cascade two enzymes using iPGA and AEH at the same time, in order to be able to increase the synthesis yield.

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9 Part IV: Pilot plant scale drug production

9.1 Pilot plant scale penicillin production

9.1.1 Introduction

Penicillin was discovered by Alexander Fleming. Different penicillins are produced by different strains of *Penicillium.*

Sodium penicillin G (MW = 356.4 KDa, Activity: 1,670 U/mg) is administered parenterally, as it is degraded in acid conditions. Penicillin is active against Gram positive bacteria by inhibition of cell wall synthesis.

Different species of the genus *Penicillium* produce different forms of penicillin. The strain used by Fleming was *P. notatum.* Later on, different strains were used, such as *P. chrysogenum,* which is the most widely used strain in industry.

The original medium contained the following compounds: lactose, 3–4%; corn steep liquor, 4% (as a nitrogen source); CaCO3, 1%; KH2PO4, 0.4%; antifoam, 0.25%. Improved media resulting in higher penicillin yields have been developed. A typical composition of such media is: glucose or molasses, 10%; corn steep liquor solids, 4–5%; phenylacetic acid (continuous feed), 0.5–0.8% total; vegetable oil-antifoam, 0.5% total. Penicillin G requires about 0.47g sodium phenylacetate per gram of produced penicillin.

The production fermenters need a mechanical agitation between (100-300 rpm) and the temperature is controlled around 25-28°C (optimum26°C)(37).

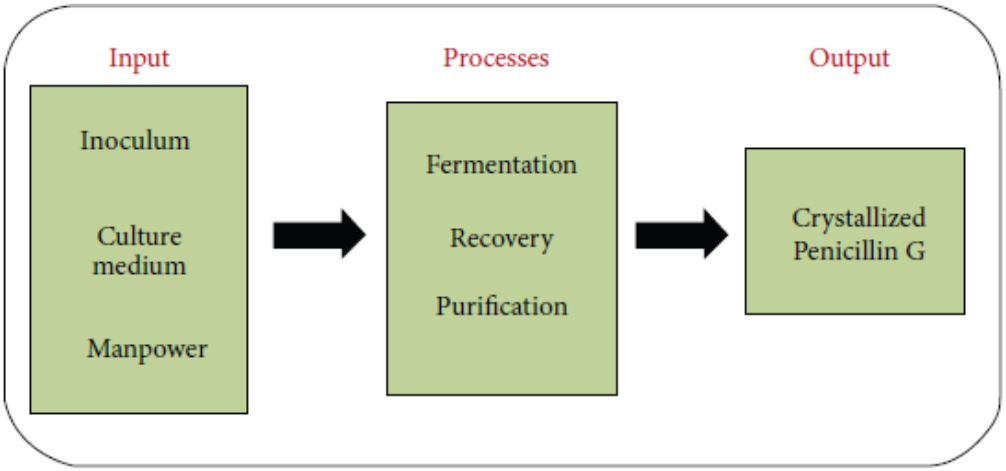


Figure 52:Schematic representation of producing crystallized Penicillin G(38).

The original process for the recovery of penicillin from fermentation broth was based on adsorption on activated carbon. After washing with water, the activated carbon was eluted with 80% acetone. The penicillin was concentrated by evaporation under vacuum at 20 to 30°C. The remaining aqueous solution was cooled to 2°C, acidified to pH = 2–3, and the penicillin extracted with amyl acetate. Penicillin was crystallized from amyl acetate with

excess mineral salts at pH of 7 under vacuum. This process is uneconomical because of the high cost of activated carbon.

The current recovery process includes filtration, extraction, adsorption, crystallization, and drying(37).

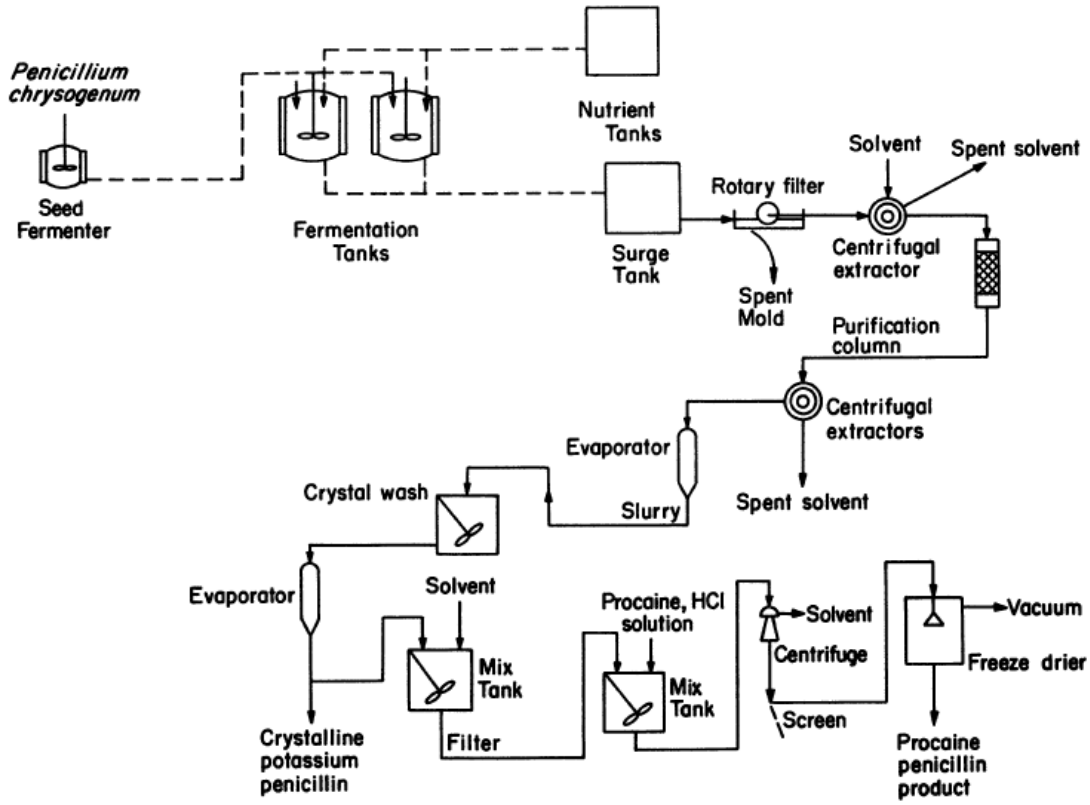


Figure 53: Schematic of penicillin production process(37).

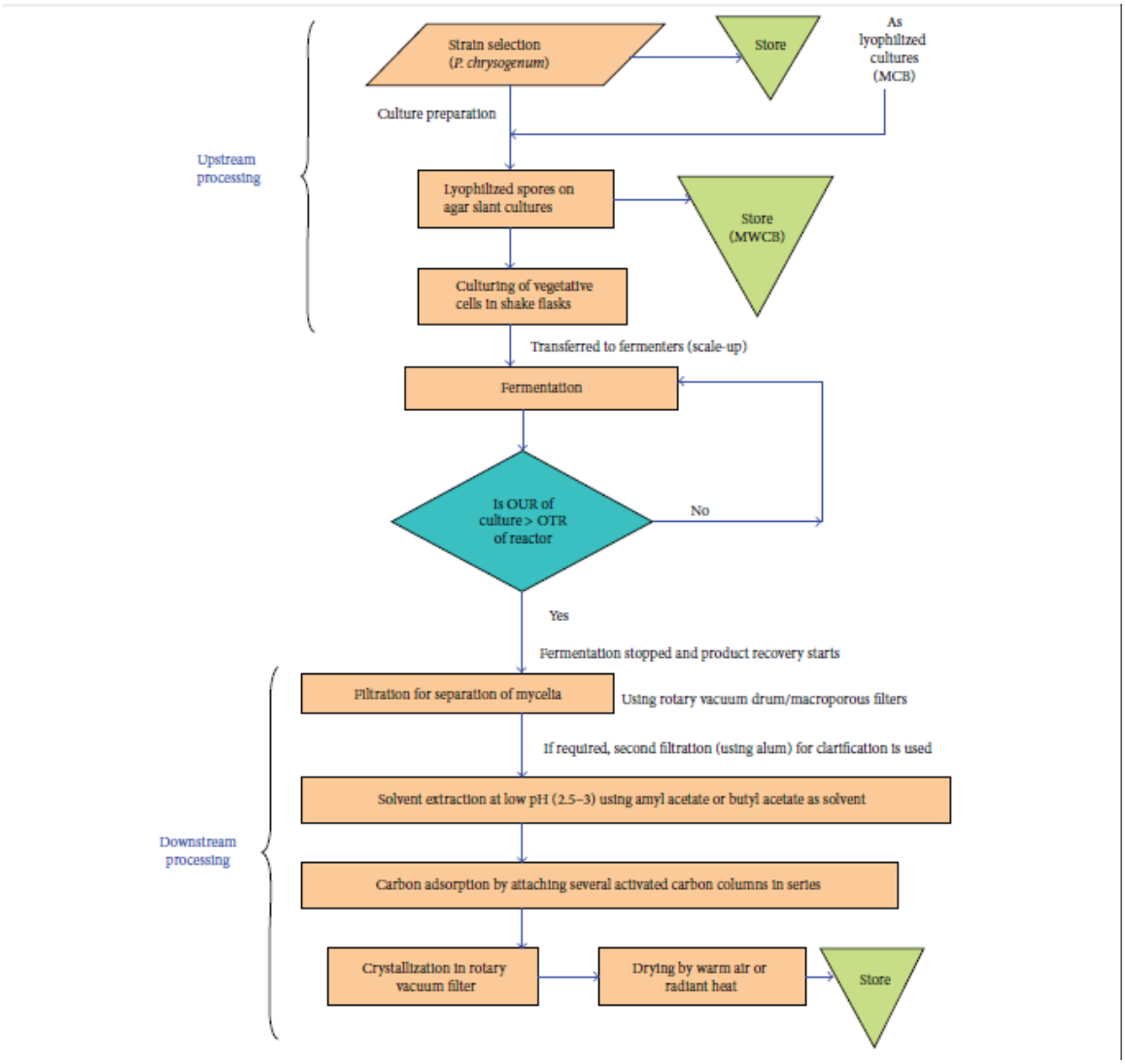
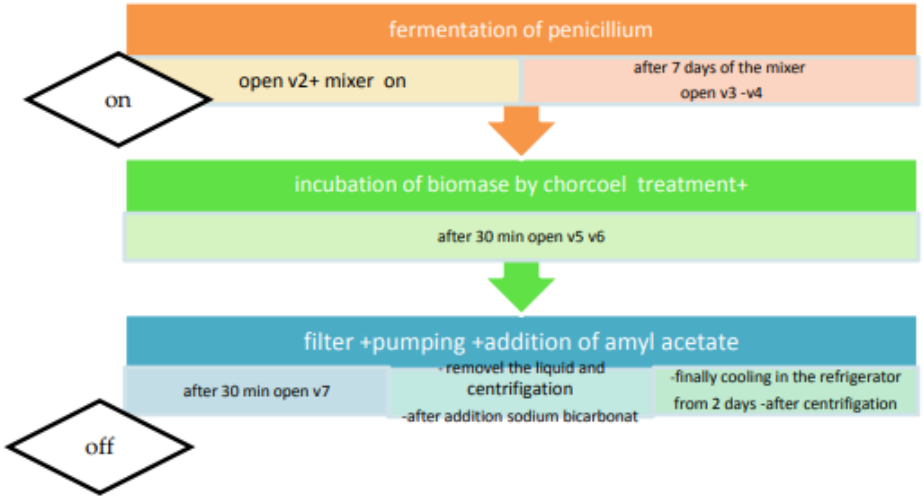


Figure 54: Schematic representation for large-scale production of Penicillin G (reproduced and redrawn from elsewhere). Steps are self-explanatory. For a detailed account, see the source. "OUR": oxygen uptake rate, "OTR": oxygen transfer rate, "MCB": master cell bank, and "MWCB": manufacturer's working cell bank(38).



time h	open valves	closed valves	mixer on/of	pump
0:00	2		on	
0:45		2		
168	3		of	
168	4			
168:30:00	5	3		
168:30:00	6	4		
169	7	5		on
169		6		

Figure 55: Program (Flow Diagram) for Automatic Synthesis of penicillin in machine(39)

Figure 56: Our current bioreactor

9.1.2 Whole study for pilot plant production with needed reagents

Table 6: pilot plant penicillin production product requirement with cost

Liquid medium	Glucose powder	400g	2.8$
	Milk powder	200g	2.75$
	Peptone	160g	4$
	Yeast extract	200g	63$
	MgCl2	30g	2$
	KCl	30g	3$
	KH2PO4	20g	4.2$
	CaCO3	40g	14.7$
	Corn oil	20g	0.1$
	Phenyl acetate	100g	28$
	Distilled water	20L	3$
Purification	Butyl acetate	1.7L	3.5$
	Sodium bicarbonate	600g	75$
TOTAL	**206$**		

This bioreactor represents the Penicillin production (15days). it is divided in 3 phases:

Microbial culture (4 days) to obtain fresh colonies of penicillium

Medium preparation (10 days)

Purification (1 day).

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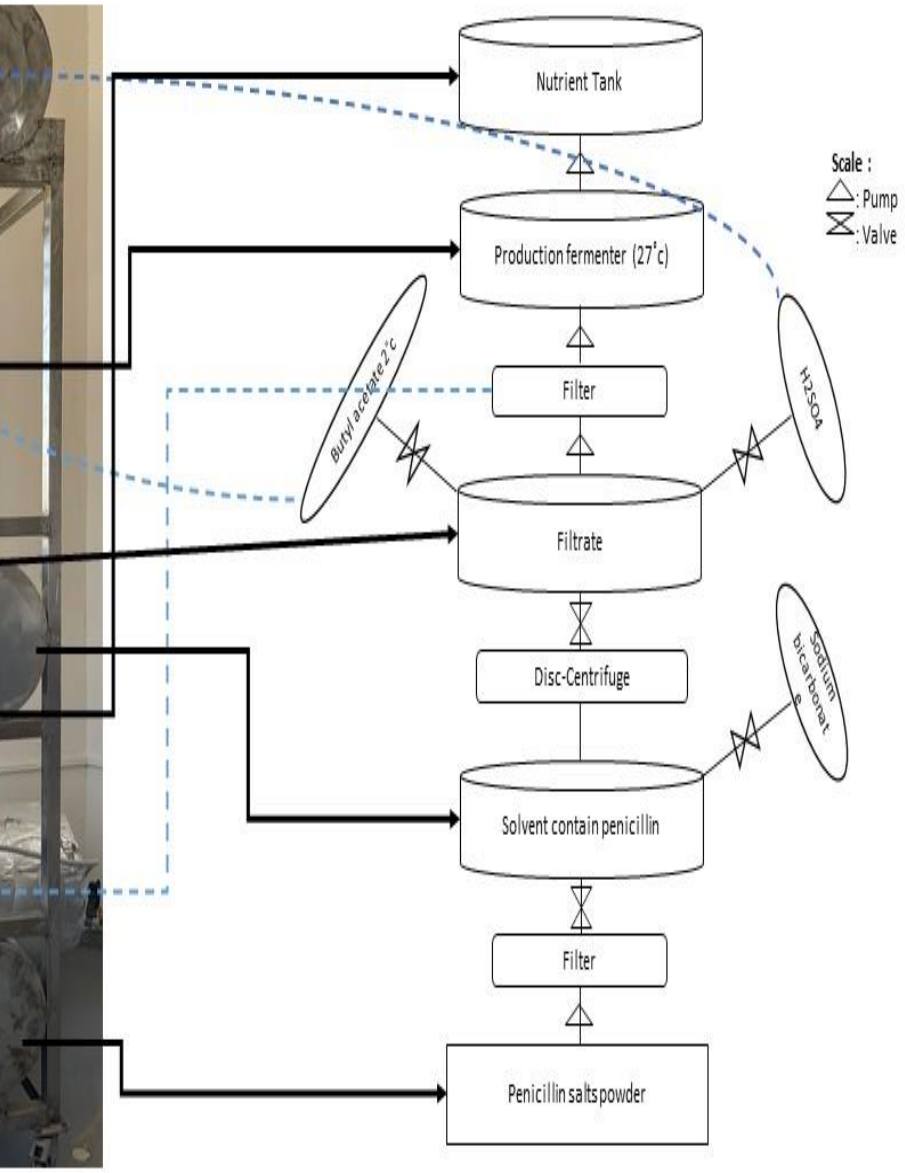

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MEGBI

PILOT SCALE REQUIREMENTS FOR PENICILLIN PRODUCTION

Introduction:

Industrial microbiology can be used to produce antibiotics via the process of fermentation, where the source microorganism is grown in large containers containing a liquid growth medium. Oxygen concentration, temperature, pH and nutrient are closely controlled. As antibiotics are secondary metabolites, the population size must be controlled very carefully to ensure that maximum yield is obtained before the cells die. Once the process is complete, the antibiotic must be extracted and purified to a crystalline product. This is easier to achieve if the antibiotic is soluble in organic solvent. Otherwise it must first be removed by ion exchange, adsorption or chemical precipitation.

1. pH sensor

pH have a good effect on controlling cell growth and antibiotic activity in batch fermentation. Tanks for H2SO4 and NaOH needed.

The UltraTough sensor and analyzer utilize our very best materials and technologies to work in the harshest of conditions.

2. Temperature prob :

Temperature variations cause changes in the rate of penicillin G production in P. chrysogenum medium. The highest rate of production was in 28°C. The production rate of penicillin was reduced significantly at temperatures above 30 ° C.

3. Disc centrifuge

Disc centrifuge can separate 2 differences liquid (different gravity). Therefore can separate the butyl acetate and filtrate of our fermentation medium.

OR

4. Podbielniak Contactor (POD)

is a horizontal liquid liquid centrifugal extractor that processes liquids for accelerated solvent extraction. The two main functions of this machine are liquid liquid separation and the liquid-liquid extraction process through counter-current flow, it can be used for pharmaceutical applications like : Various antibiotics such as Penicillin G, ect.

5. Crystallizer

Batch crystallization is a method for polishing antibiotics, including penicillin G. Batch crystallizers simply consist of tanks with stirrers.

They are slowly cooled to produce **supersaturation**. Seeding causes nucleation and growth is encouraged by further cooling until the desired crystals are obtained.

6. Crystal washing

While the penicillin G crystals we have formed are essentially pure in nature but adsorption and capillary attraction cause impurities from its mother liquor on their surfaces and within the voids of the particulate mass. Because of this the crystals must be washed and pre-dried in a liquid in which they are relatively insoluble. This solvent should be miscible with the mother solvent.

7. Drying of crystal

• **Vacuum Band Dryers:**
A thin wet layer of penicillin crystals are fed onto a slow rotating heated drum. Radiant heat dries the layer and scalpels remove the product from the end.

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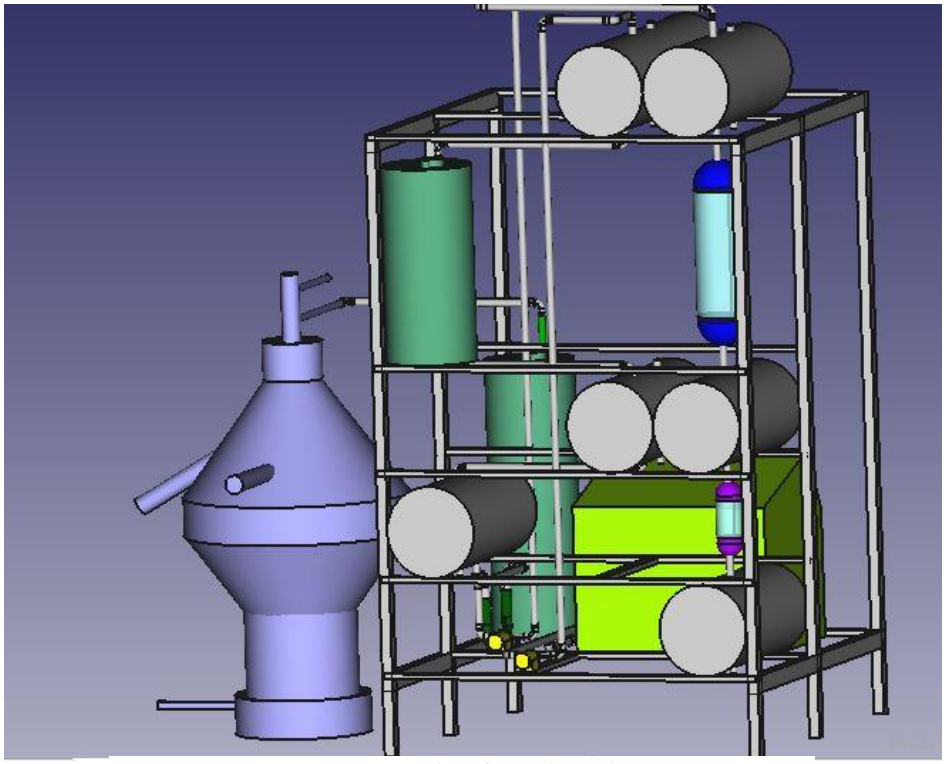
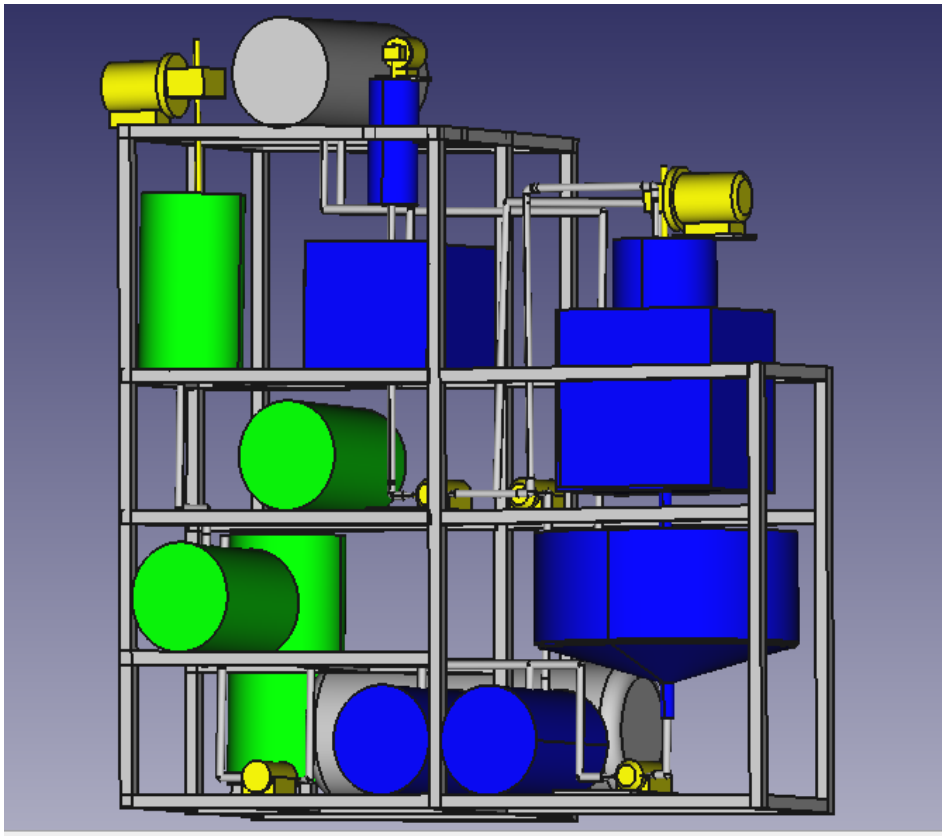


060116MEGBI-VPP_
Assembly_simplified

Figure 57: FreeCAD MEGBI pen/amp/asp pilot scale version 2015



aspirin
filtration0.FCStd

aspirin filter +
crystallizer.FCStd

Figure 58: MEGBI penicillin/ampicillin/aspirin pilot scale production new design.

Blue tanks : ***Aspirin.*** ***Green tanks :*** ***Penicillin/Ampicillin***

9.2 Pilot scale test specification

9.2.1 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

Make sure all valves are closed

Make sure the power is turned off

Connect the water valve to autoclave

Open the water to fill the tank (amount of water should be between 60-70%)

Closed 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 in water

3-Plug the control system

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

5-Operate the heater

6- Wait till the water transform to steam, operate the valve system to open

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

9.2.2 System testcases

9.2.2.1 001: test the resistor of the autoclave

Step	**Step Description**	**Expected Result**
Precondition	System is off	

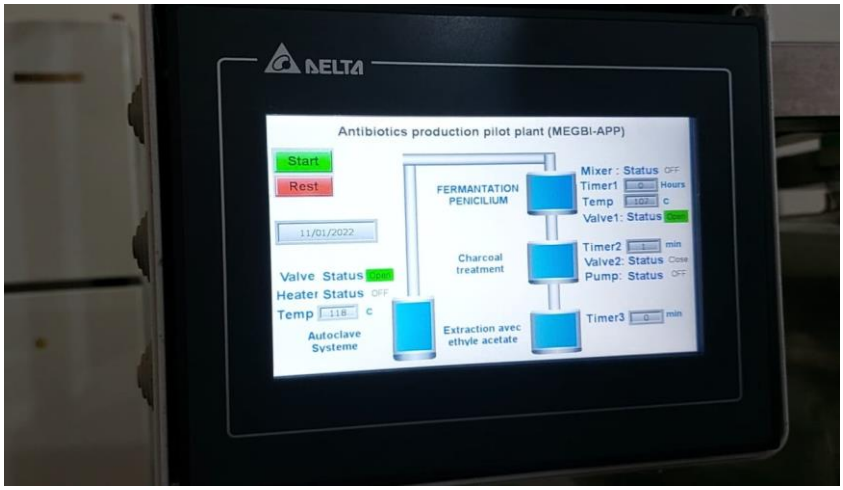
Switch on the system	Turn on the heater	The water degree starts to going up to reach 121°c
Switch off the system.	Turn off the heater	The water will remain warm and could last for 5-6h
Postcondition	System is off	

9.2.2.2 002: WHOLE SYSTEME TEST

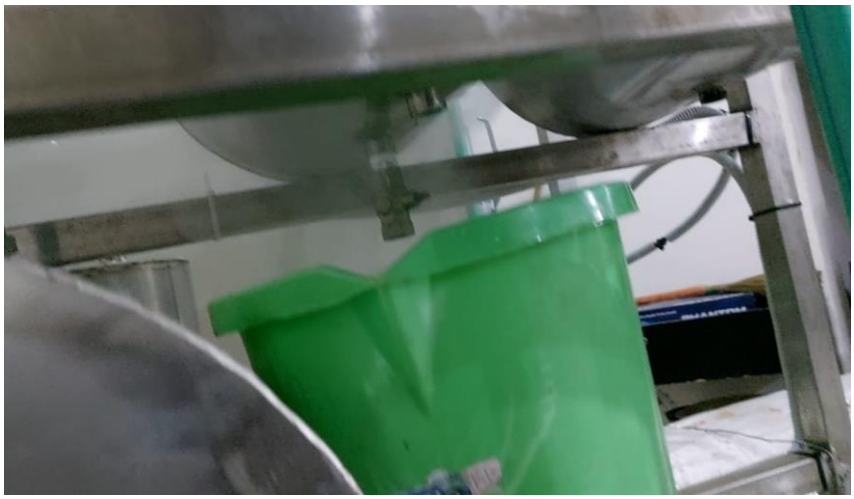
Step	**Step Description**	**Expected Result**
Precondition	System is off	
open the valves	open the valves from the control panel	Release the air from the system
Switch on the system	Turn on the system from the control panel	THE SYSTEM IS heating the water till reach 121°c
Sterilization the fermenter	Open the fermenter valve	The steam is filling the fermenter
Sterilization the whole system	Open the last valve to sterilize last 2 tanks	Pressure should reach 2 atm and temperature 121 °c
Open the exit pipe	Operate the exit pipe to open through the control panel	Pressure should decrease and the steam vent off the system
Postcondition	System off the system	system is off

1-Turning on the system through the Control panel till reaching the expected temperature and pressure.

2-Opening the Valves (fermenter and purification tanks) to complete the sterilization test.



3-Opening the manual valve to insure the steam is exist.



video autoclave test part 1 (http://aecenar.com/index.php/downloads/send/6-megbi-institute/1169-test-video-part-2-1)

10 Part V: Ministry of health licence

Lebanon chose to follow the "GUIDELINES ON EVALUATION OF SIMILAR BIOTHERAPEUTIC PRODUCTS (SBPs)" issued by the WHO, as adopted by the 60th meeting of the WHO Expert Committee on Biological Standardization, 19-23 October 2009 to evaluate the submitted SBS Files.

. Guidance For Registration Of Similar Biological Medical Products (Biosimilars) pdf

. List of Requirements for the registration of Biosimilar products according to CTD format pdf

According to these files and in order to register for a similar Biological medical product a Reference biotherapeutic product(RBP) is used as the comparator for head-to-head comparability studies with the Similar biotherapeutic product(SBP) in the aim of showing similarities in terms of **quality, safety and efficacy**.

Only an originator product that was licensed on the basis of a full registration dossier can serve as a RBP.

The technical file for Registration of a Locally Manufactured and/or packed under License Generic Chemical Product, shows a list of studies needed including **Methods of analysis , stability data, storage conditions, bioequivalence...**

Technical file for registration doc

The Quality module for the drug substance defining the validation parameters needed for a variety of analytical methods of control and describing characteristics to be considered for the validation of analytical procedures are included in a marketing authorization application (MAA).

This document is intended to provide a global policy and guidance for the preparation of the Quality module of Drug Substance for an application file that meet with the requirement of Ministry of Public Health in Lebanon.

Guide for the quality module3

According to the quality guide module, an **analytical procedures** used for testing the drug substance should be provided. The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:

- Identification tests
- Quantitative tests for impurities' content
- Limit tests for the control of impurities
- Quantitative tests of the active moiety in samples of drug substance

As MEGBI research Center in AECENAR our goal is to produce local drugs that meets international safety standards according to the recommendations of the Ministry of Health .

-→ Starting with **Aspirin**,

here we can find the essential analytical Procedures for standard quality tests that we can use to compare our crude aspirin.

Specifications and Test Procedures (ASPIRIN-USP) .pdf

Specifications and Test Procedures (ASPIRIN-BP) .pdf

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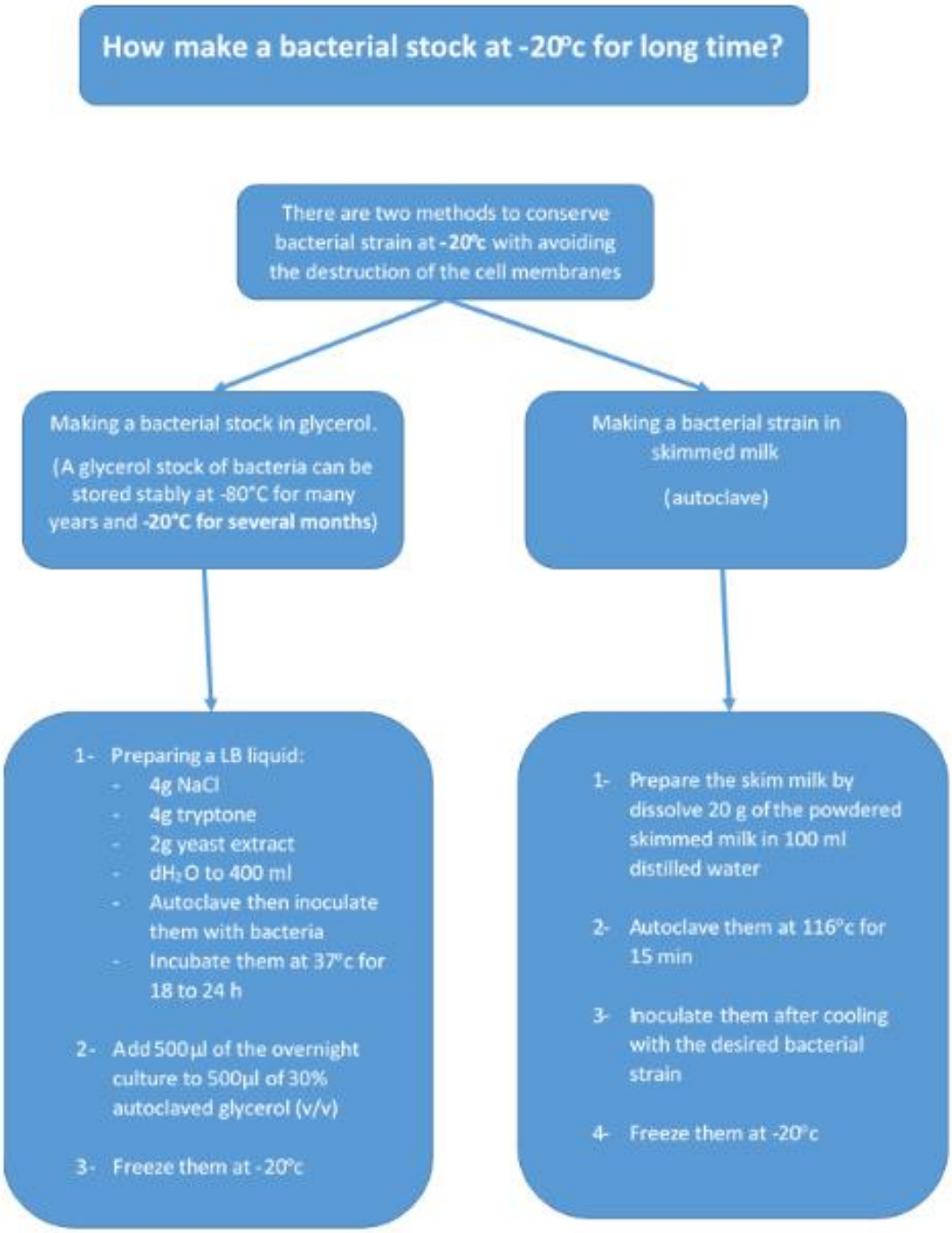
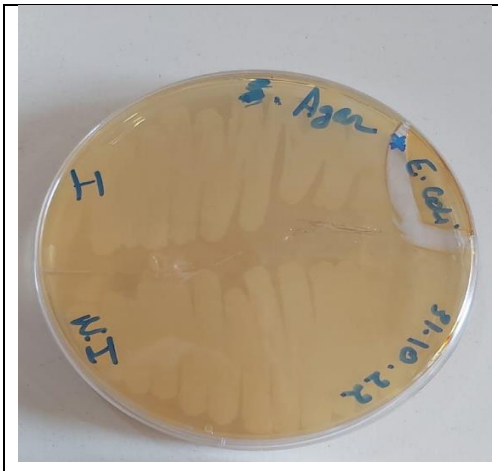
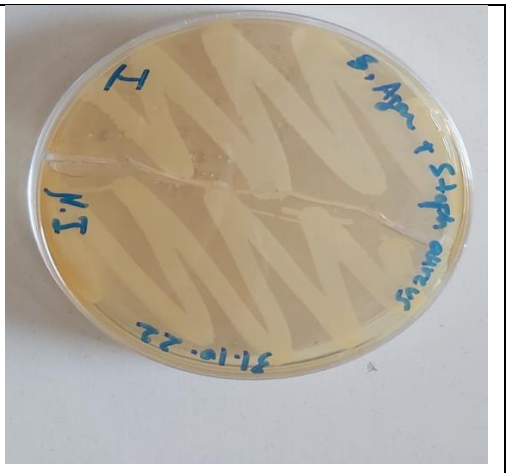
12 Annex: The most important solutions in biology lab

12.1 Making bacterial stock solution



12.1.1 Realisation: 28-10-2022





The strain has to be fresh when it shall 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

12.2 Solutions preparation

12.2.1 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).

12.2.2 H_2SO_4(1M)

- Fill the flask half way with distilled water
- Add 56 ml of H_2SO_4
- add water to reach the 1L mark
- Finally mix it to obtain H_2SO_4 solution (1M).

12.2.3 NaCl 0.9%

- We put 0.9g NaCl in 100 ml distilled water
- Put it in Autoclave for microbial usage.

12.2.4 Bleach 1%

-We took 1ml of bleach solution

-Put it in 100ml water graduated cylinder

-Mix it to obtain Bleach solution 1%.

12.2.5 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.

12.2.6 Standard agar:

-Measure 5.55g of standard agar 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.

12.2.7 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.

12.2.8 Phosphate buffer (0.1M, pH:7)

-Prepare 800 ml of distilled water in a suitable beaker

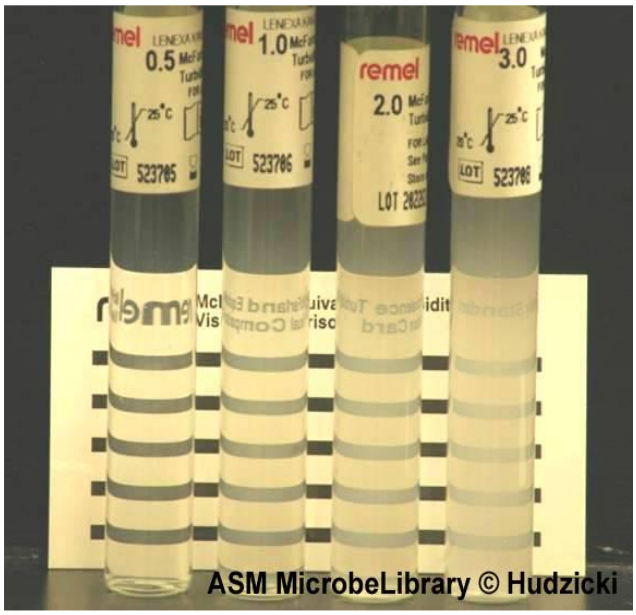
-Add 9.34g of Potassium phosphate dibasic (0.05364M, mw: 174.18g/mol) to the solution

-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.

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



- we added 0.5 mL of a 0.048 mol/L solution of $BaCl_2$ (1.175% w/v $BaCl_2$ $2H_2O$) to 99.5 mL of a 0.18 mol/L solution (0.36 N) of H_2SO_4 (1% v/v) and we shook vigorously
- 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

- We distributed the suspension in tubes of the same size as those used to adjust the inoculum and then we sealed the tubes
- 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)

12.2.10 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$

12.2.11 MEGBI-Suppliers 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	

بسم الله الرحمن الرحيم

MEGBI - Middle East Genetics and Biotechnology

MEGBI-APP (Antibiotics/Aspirin Pilot Plant) Report 2023

- **Penicillin and Ampicillin production and quantification (Lab scale)**
- **Aspirin production and quantification (Lab scale and Pilot Plant Scale)**
- **Generic Pilot Plant Design for Penicillin/Ampicillin/Aspirin Pilot Plant**

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Last Update: 30.12.2023 14:14

Edited on 7.4.2025 by Dr. Samir Mourad

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 consummer to treat even to prevent several diseaes. 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 medecines like these mentionned above, for this reason the import still the ideal solution to secures the locally need. According that, we as AECENAR, and specially as MEGBI institute, we try to find and even to produce these medecines locally by developping a bioreactor which can produce this types of drugs.

13 Part I: Aspirin Lab Scale Production and Qualification

13.1 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 comparision	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	
Sulphated ash	Silica gel					crucible, muffle furnace, desiccator	
Assay	Phenolphthalein	As an indicator	50 g	$44.84	VWR		
Total				$680.80			

13.2 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 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 point apparatus		$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	
	Na2SO4	1 kg	$17.00	VTC	
Total			$1,220.00		$1,220.00

13.3 Aspirin Optimization

13.3.1 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.

13.4 Results

Trial	Volume	Molarity of Acid	Recrystallization solvent	Reaction time	Mass of Product	% Yield
1	5 mL acetic anhydride	1M (sulfuric acid)	warm water	10 min 10min 10min 15min 15min	1.96g - - 1.56g 1.21g	75.1% 60% 46.3%
2	5 mL acetic anhydride	6M	water	10 min	1.60g 2.42g 1.07g	61.3% 92.7% 41.15%
3	5 mL acetic anhydride	6M	ethanol and water ethanol and cold water ethanol and warm water ethanol and warm water ethanol and warm water ethanol (10ml) and warm water ethanol 5%, distilled water at room temperature ethanol (10ml) and warm water	10 min 15 min 10min 15 min 15min 15min 15min 15min	0.85g 0.68g 0.88g 0.37g 0.94g 0.65g 1.28g (unkown residue milky-oily mass) 2.08g (98% concentrated acid)	32.6% 26.6% 33.7% 14.1% 36% 24.9% 49.1% 79.6%
4a	1.56 mL acetic anhydride 1.29 mL acetic acid	1M	water	10 min	1.85g	70.9%
4b	1.56 mL acetic anhydride 1.29 mL acetic acid	6M	water	10 min	2.37 g	90.8%
5	5 mL acetic anhydride	6M	water	2 hr	-	-

6	5 mL acetic anhydride	6M	water	20 min	2.39 g	91.5%
7	1.56 mL acetic anhydride 3.44 mL acetic acid	6M	water	10 min	1.57g	60.2%
8*	5 mL acetic anhydride	6M	water	10 min	1.80g	69.0%
9	5 mL acetic anhydride	6M	Room temp distilled water	10 min	2.97g	114%
	4 ml acetic anhydride	6M	- ()	15 min	1g	40%

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.

13.5 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:

nacetic anhydride = 1.18 nsalicylic acid = 1.18 x 2 g / 138 g/mol = 0.0165 mol

Vacetic anhydride = n x MM / d = 0.0165 mol x 102 g/mol / 1.08 g/mL = 1.56 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.

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

13.6 Conclusive Remark

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 (135oC), as well as the melting point of medicinal aspirin (~130oC). 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

14 Part II: Penicillin Lab Scale Production and Quantification

14.1 Penicillin Production and Quantification May2023

Trial 2:

according to the sensitivity test :

_F1(Aqueous phase after the organic solvent extraction)

_F2(Organic phase After PB extraction)

_F3(the final solution obtained after organic solvent extraction)

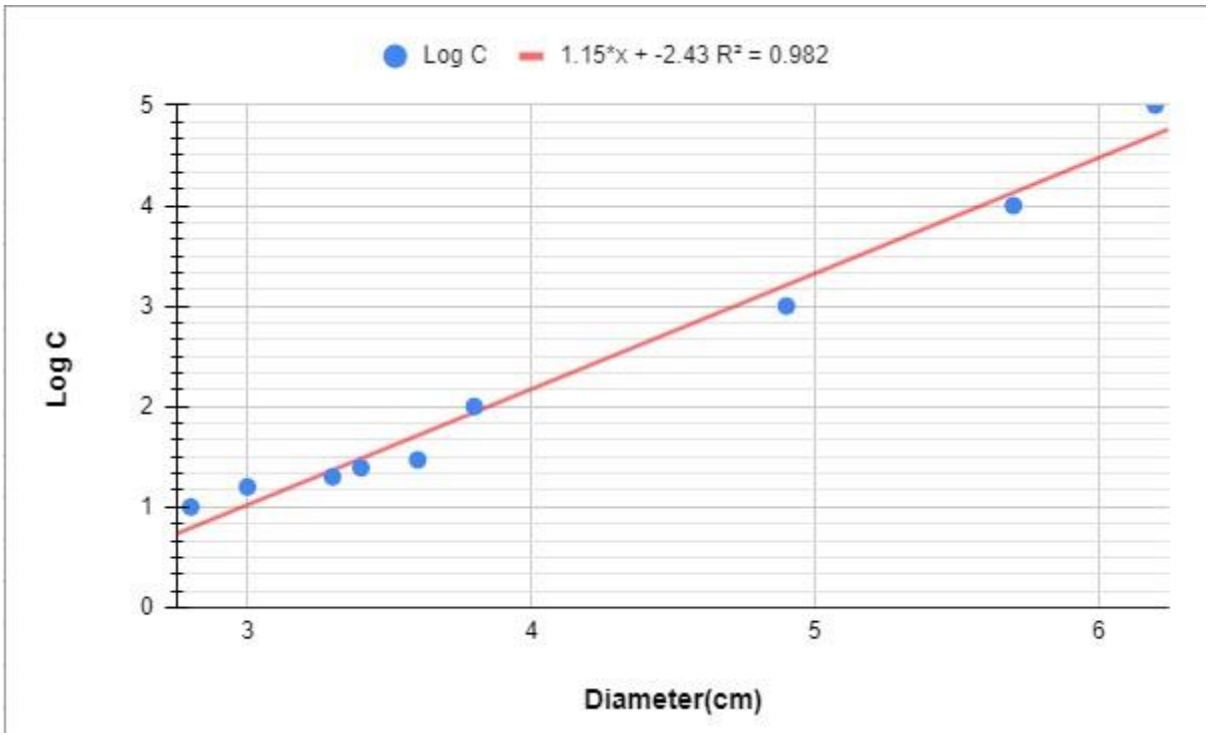
14.1.1 Penicillin Quantification (May 2023)

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:

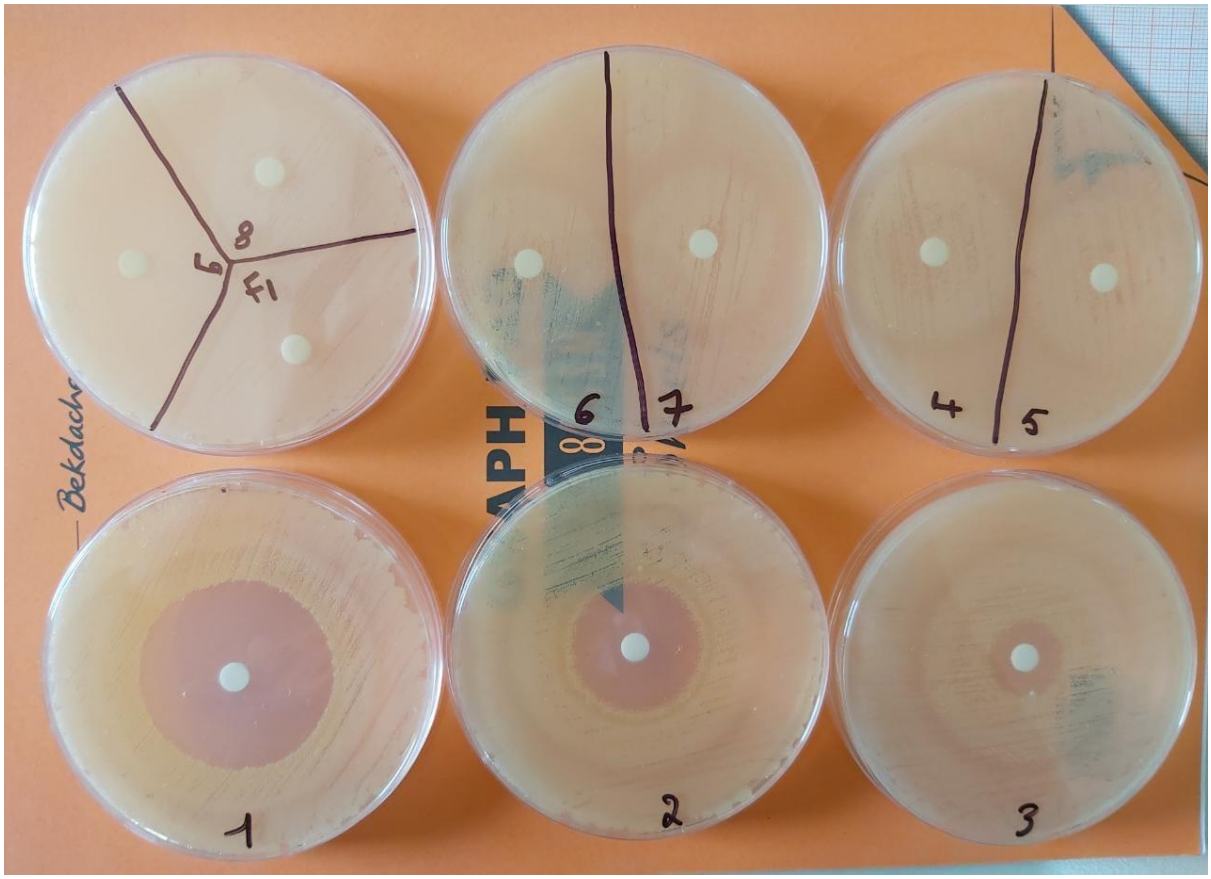
Quantification of Crude PenG obtained from F1 crystallization:

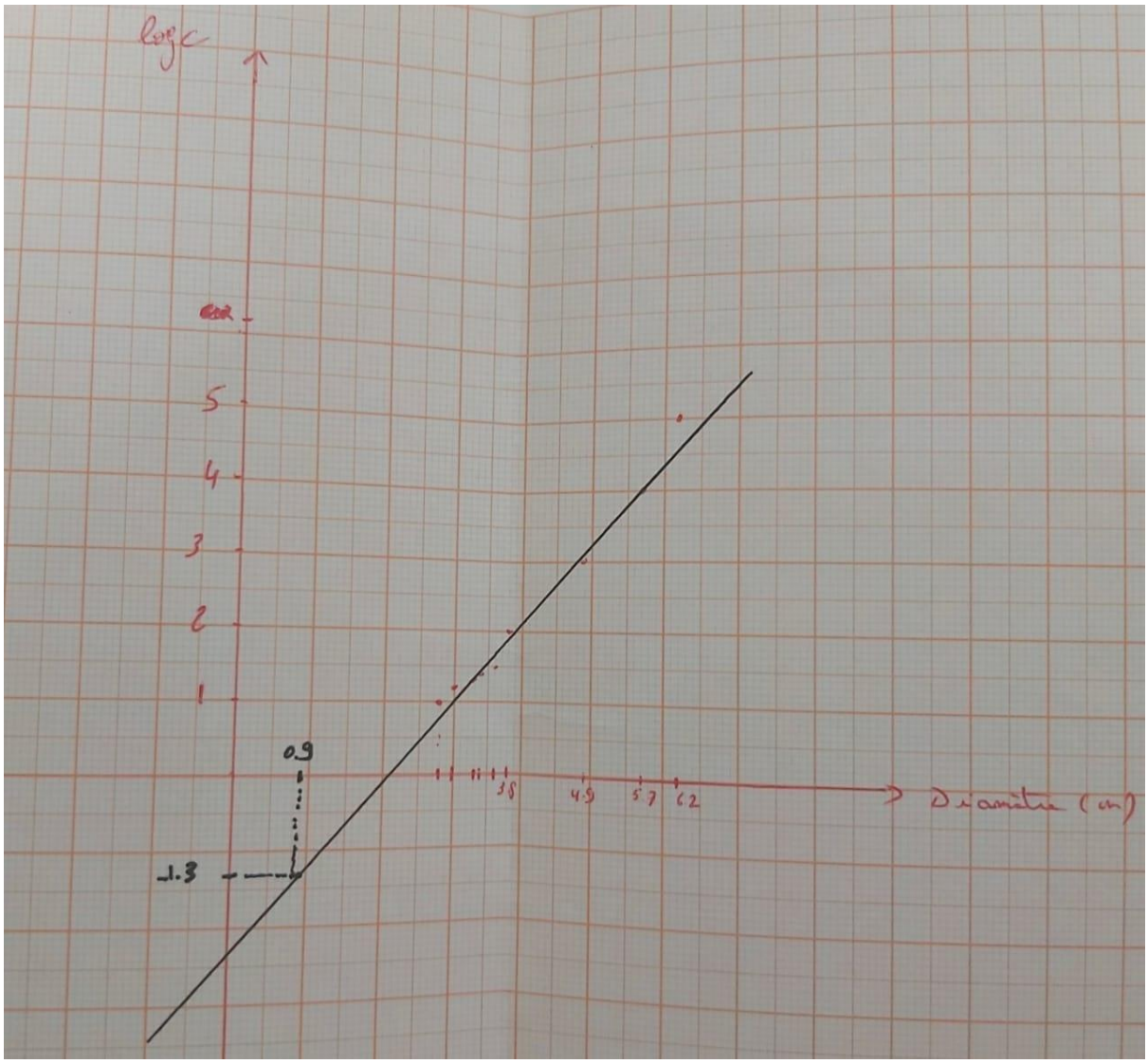
Table1 : Measurements of diameters of bacterial growth inhibition zones for different concentrations of standard dilute commercial penicillinG and F1.

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





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

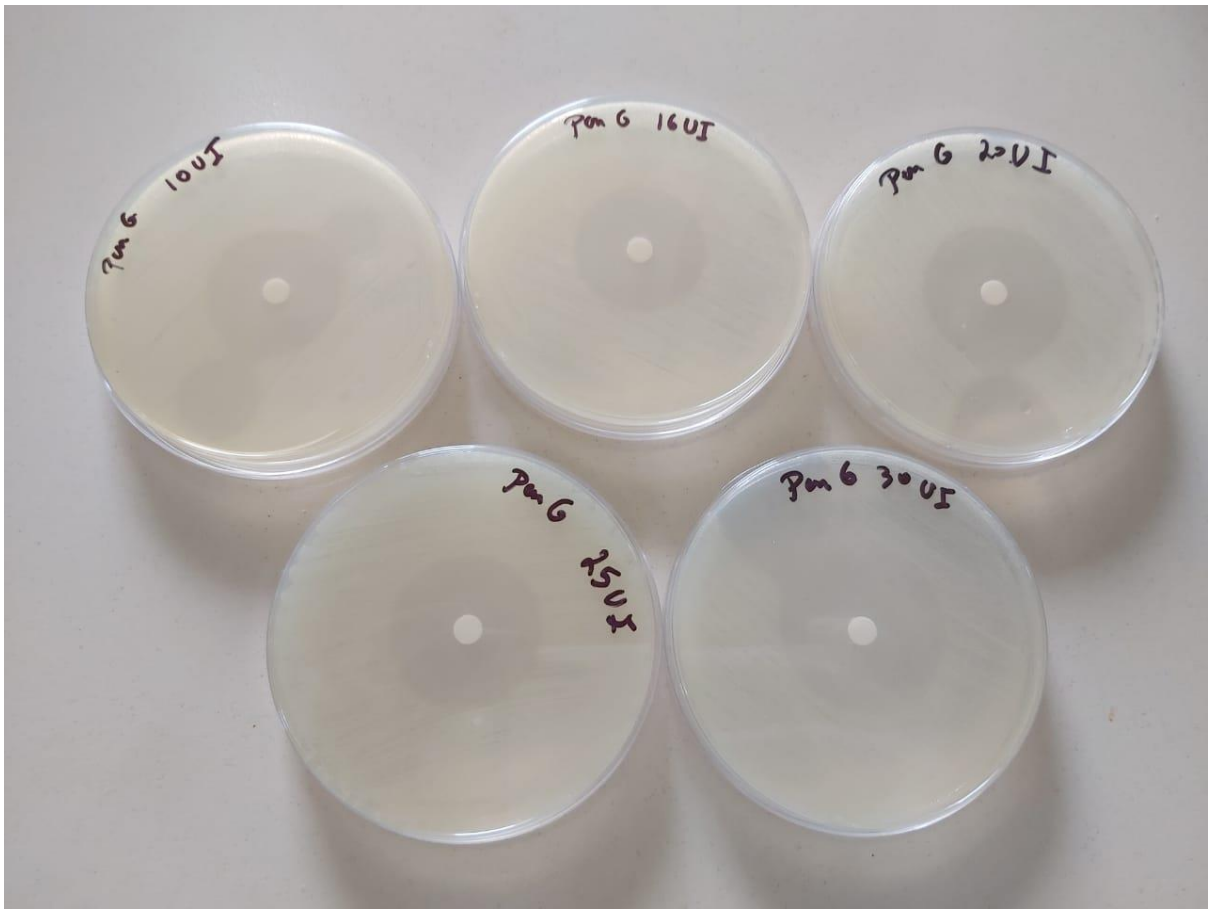
So the concentration of PenG in F1 is 0.04mg/ml

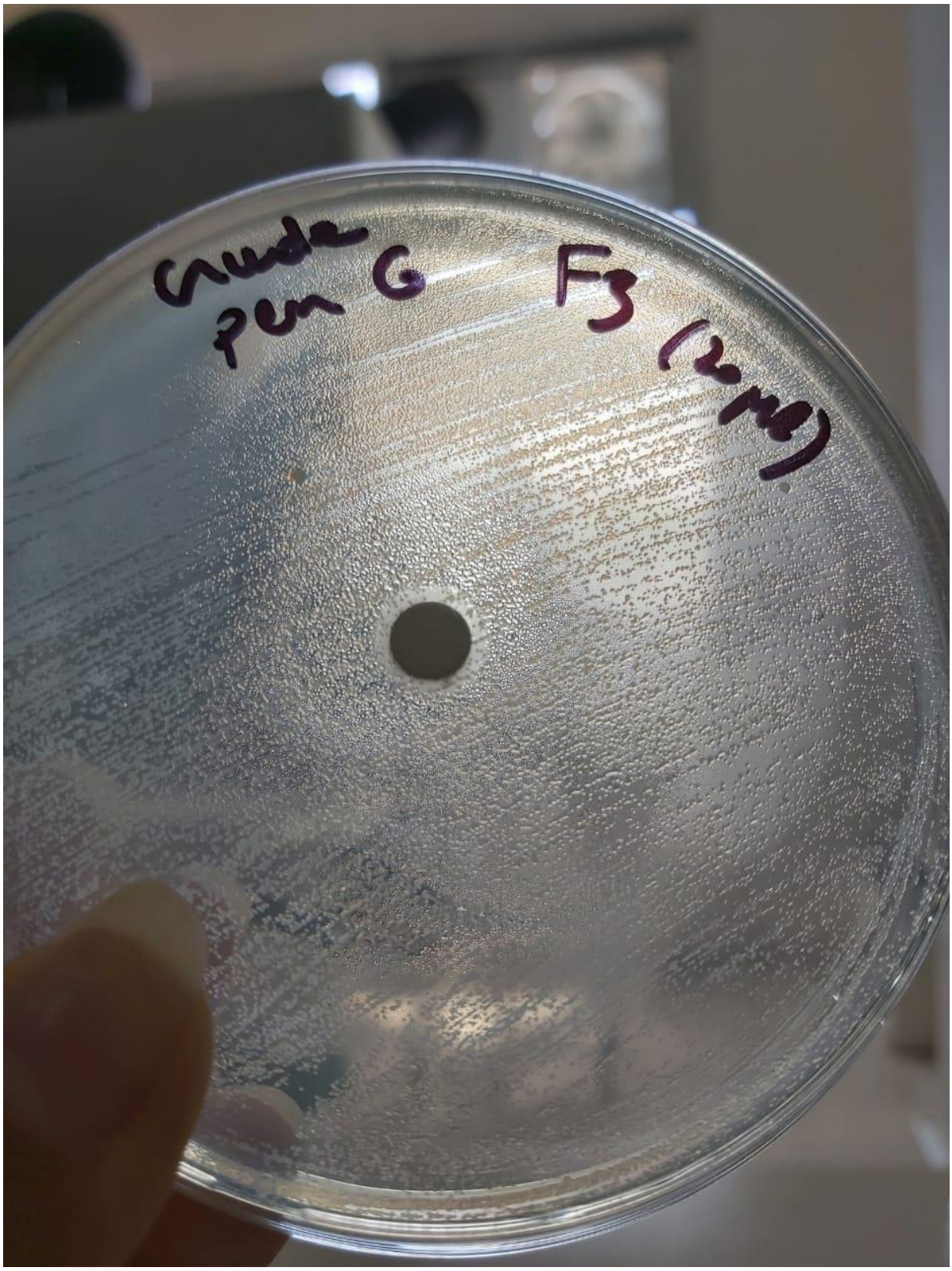
Quantification for Crude PenG obtained from F3 crystallization

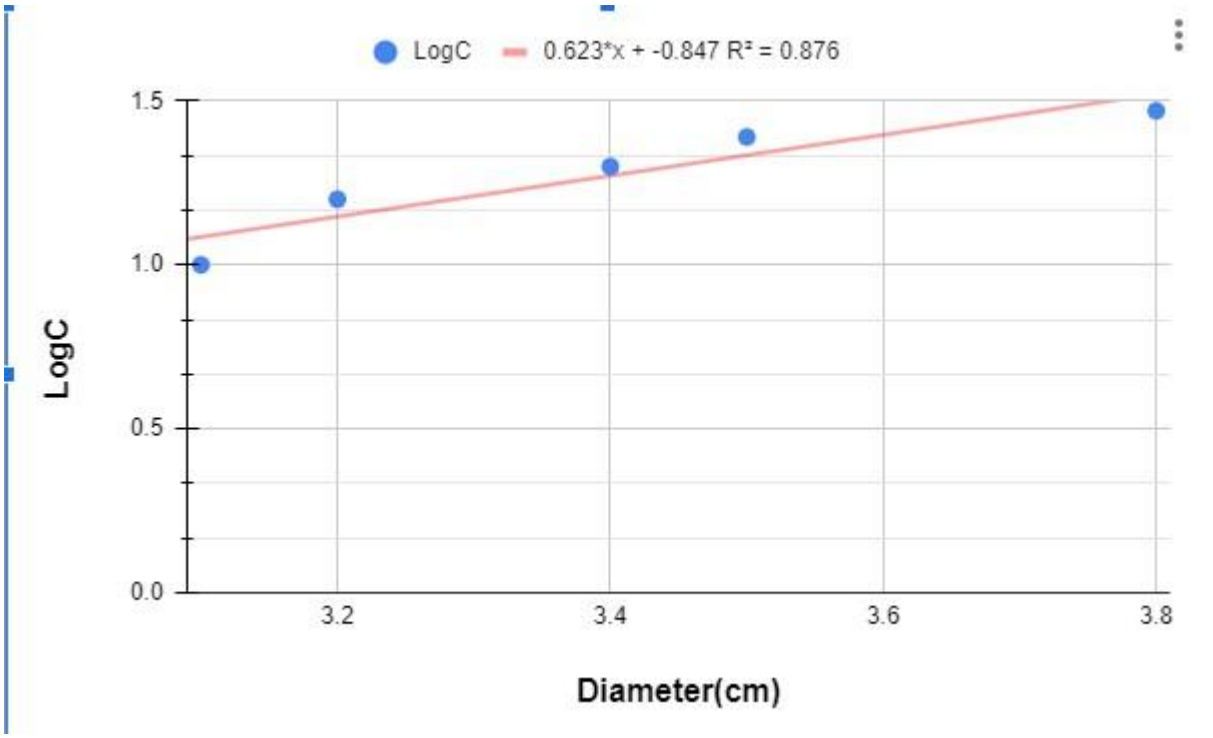
Table 2 : Measurements of diameters of bacterial growth inhibition zones for different concentrations of standard dilute commercial penicillinG and F3 solution.

Diameter(cm)	3.8	3.5	3.4	3.2	3.1	0.7

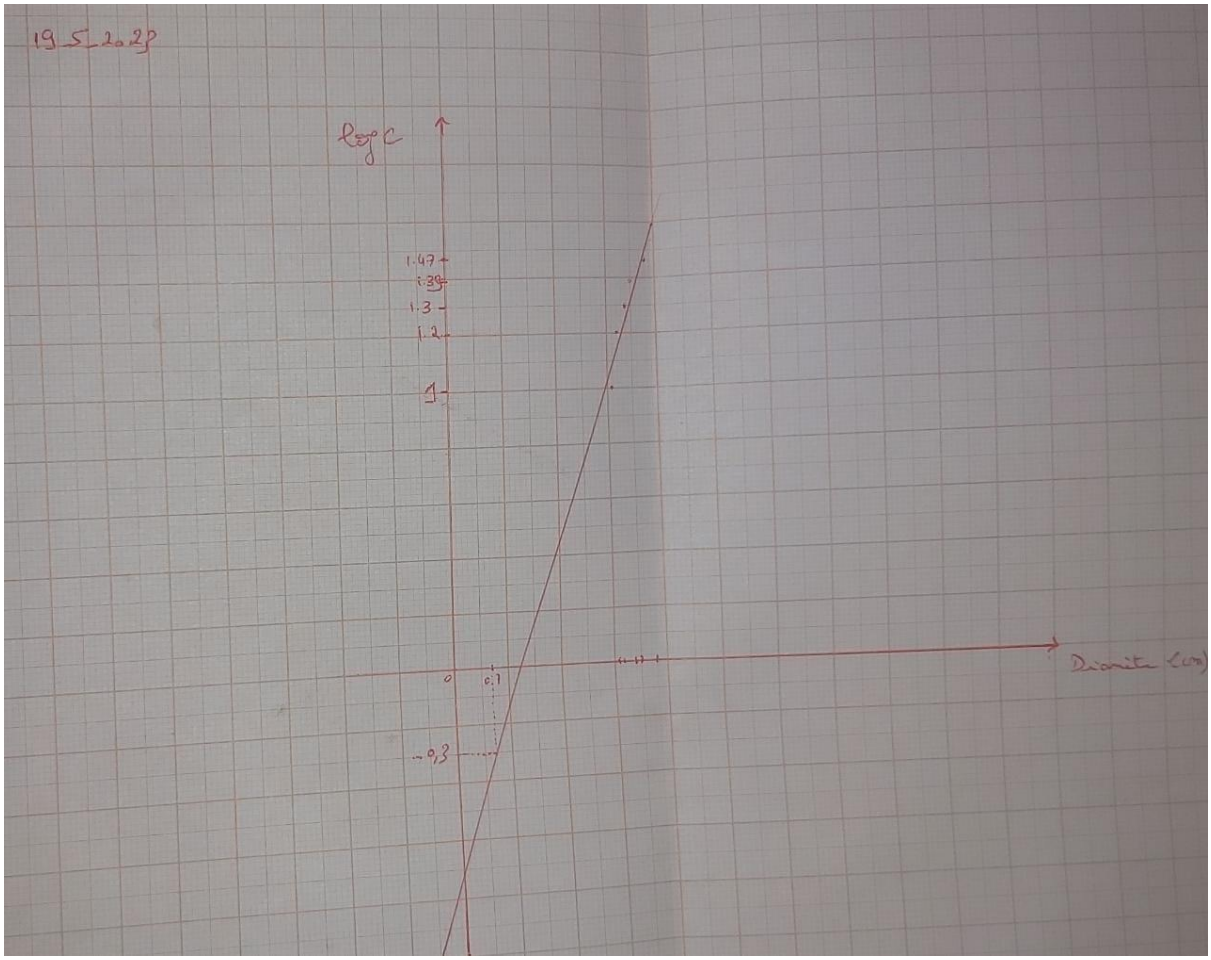
LogC	1.47	1.39	1.30	1.20	1	?
Concentration(mg/ml)	30	25	20	16	10	




Crude
Pen G
F3
(2 ml)



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



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

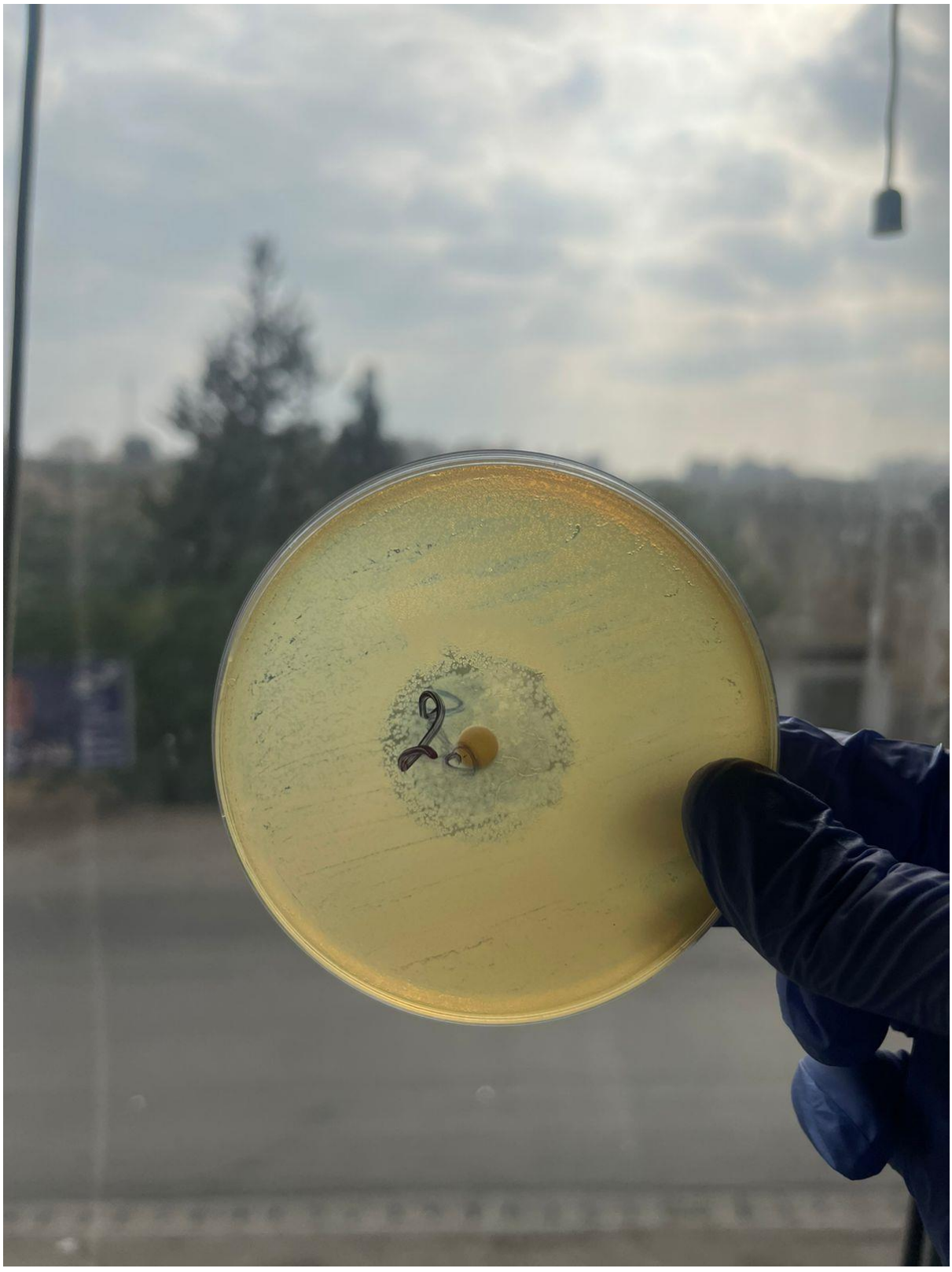
Steps of preparation	Working Date	Duration(Days)
Penicillium culture	April 19	4 days
Fermentation	April24 to May3	10 days
Purification: filtration	May4 to May5	2 days
Crystallization	May5 to May12	7 days
Sensitivity test	May15 to May16	2 days
Quantification/results F1	May15 to May16	2 days
Sensitivity test Quantification/result F3	May18 to May 19	2 days

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 temprature to 25˚C for the rest of the fermentation).

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

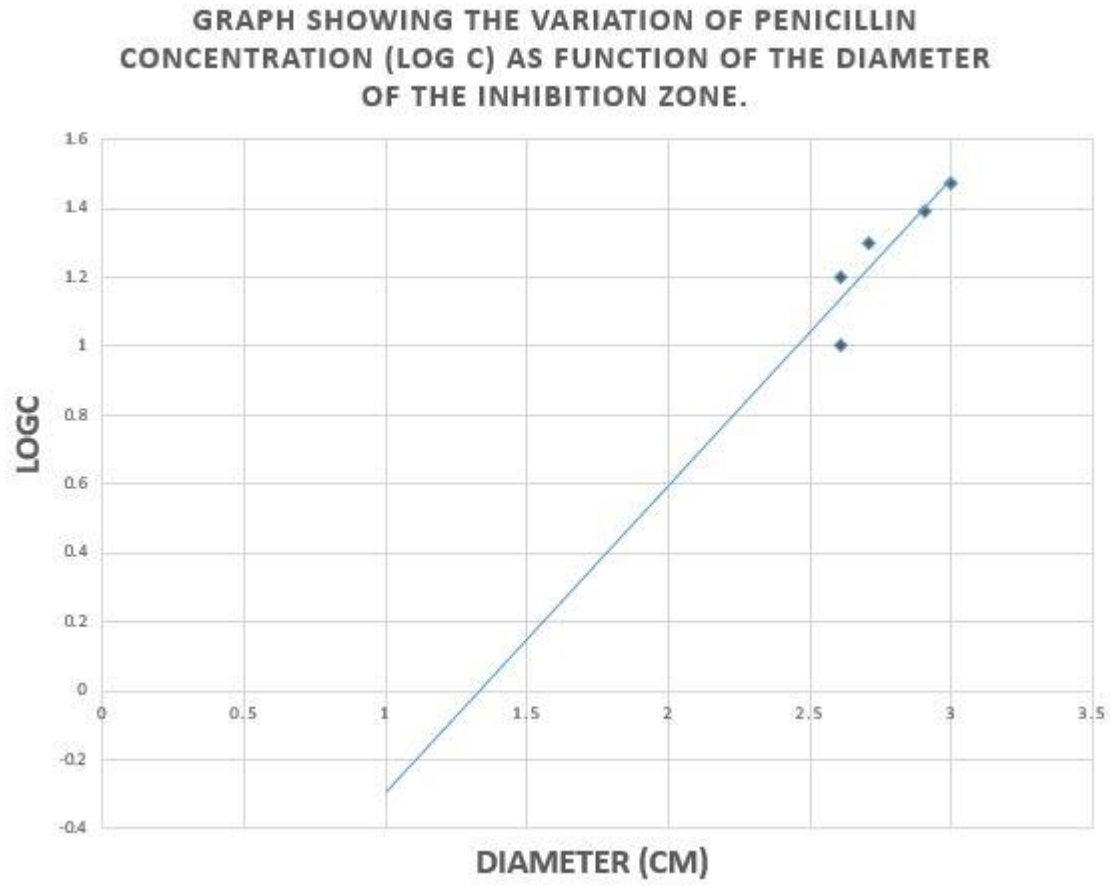
14.2 Results




25
10

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**



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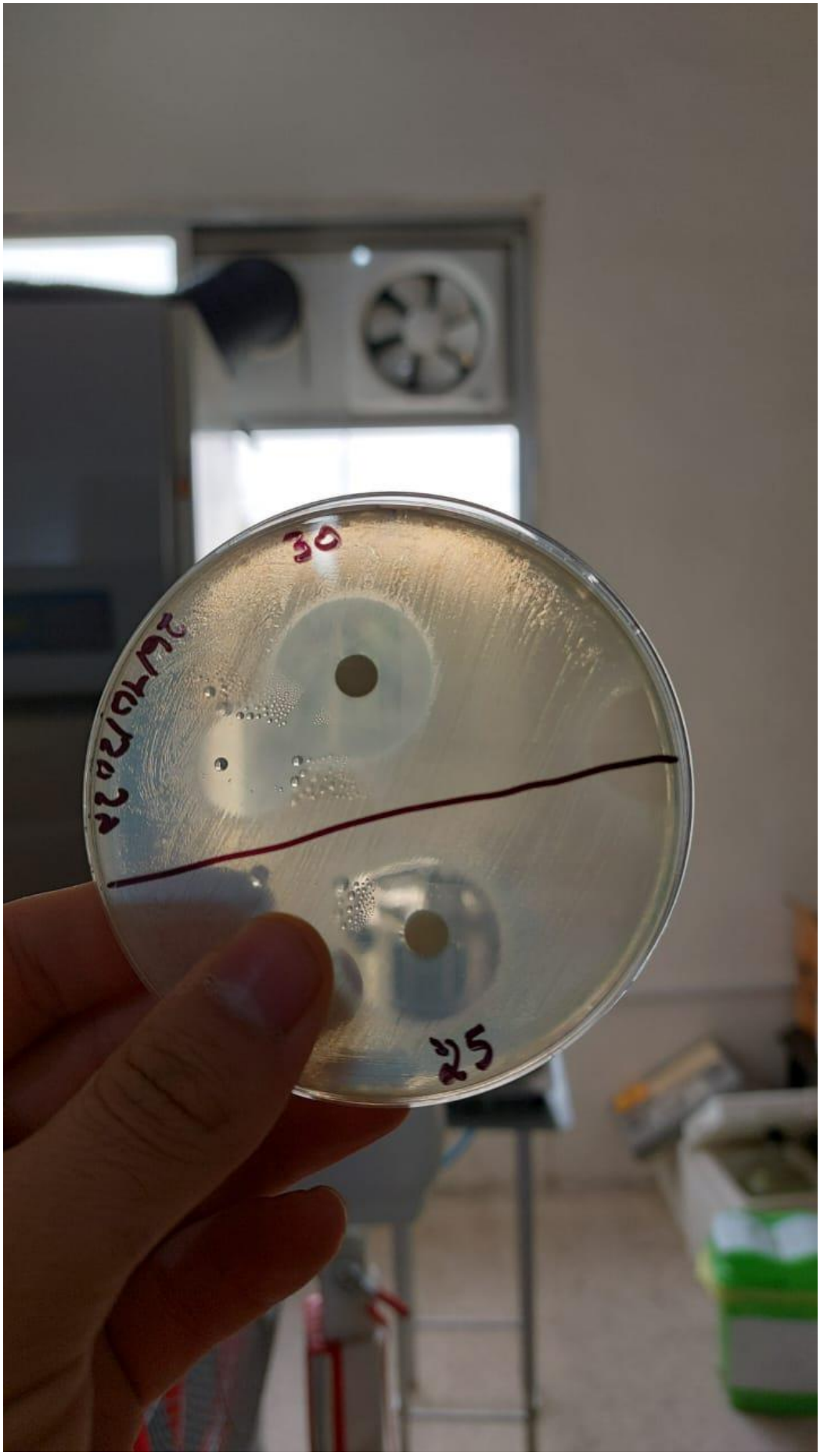
trial 4

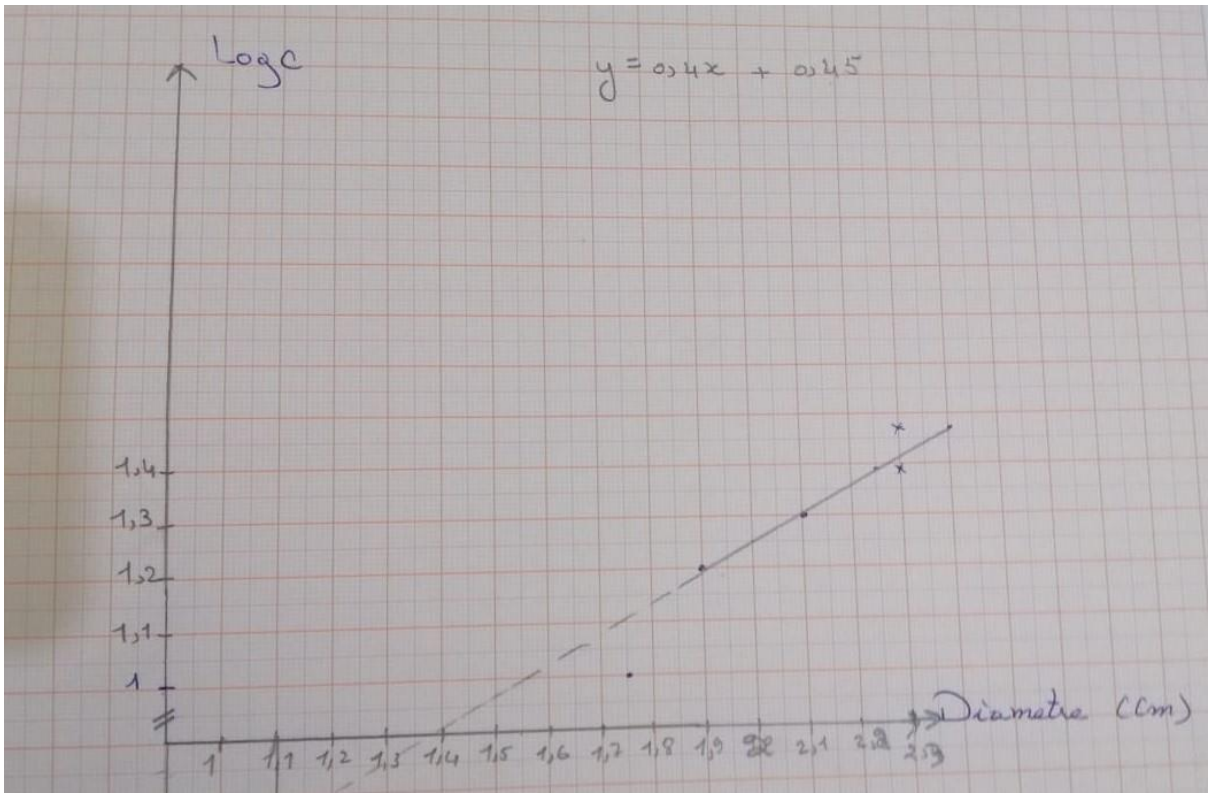
Same protocol reused in this trial (except:aeorbie respiration and medium: without glucose , lactose 3g).

14.2.1 Results




15
20

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.

15 Part III: Ampicillin Lab scale Production

15.1 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-(-)-a-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)

Ampicillin purification and harvesting:

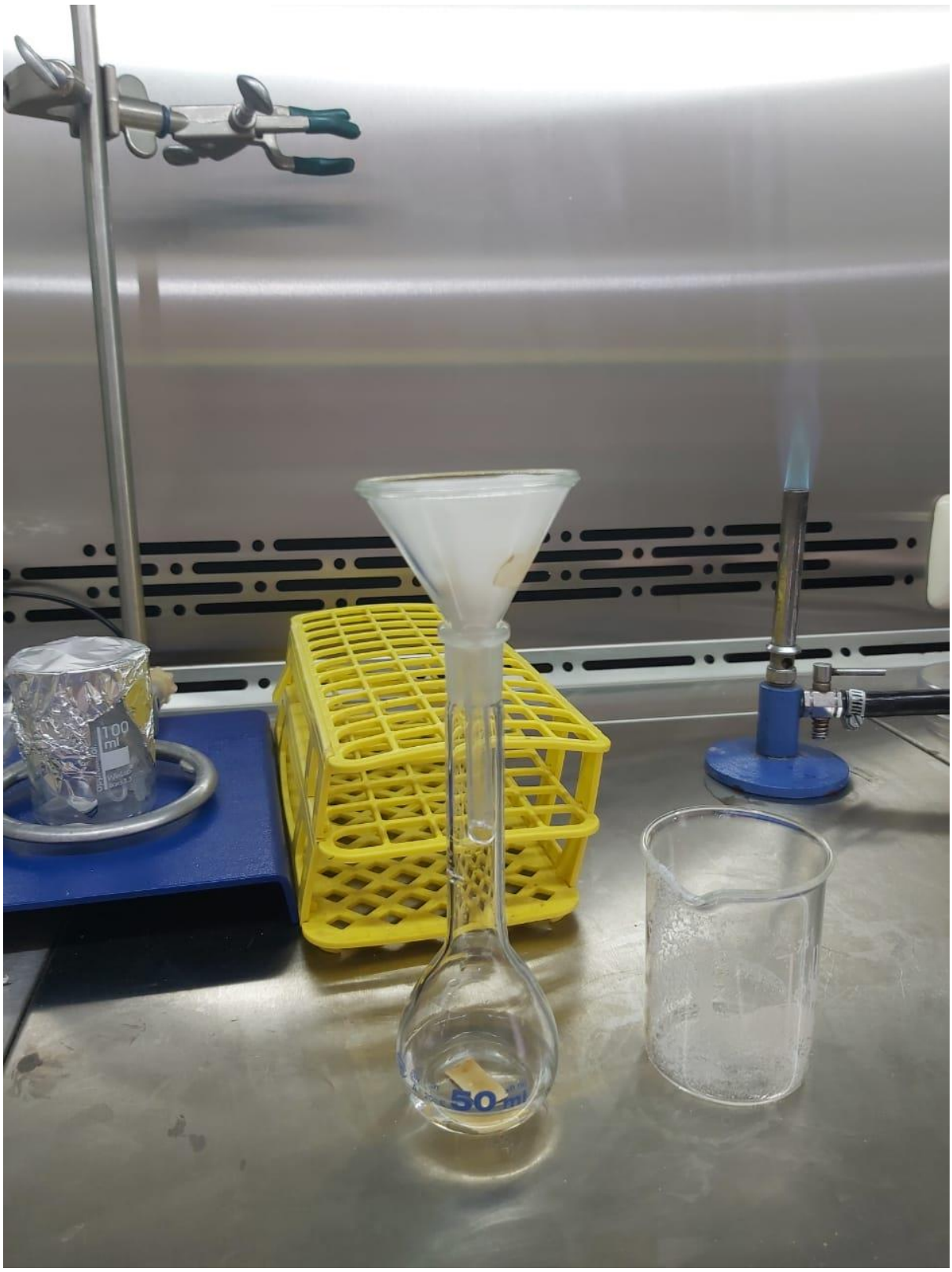
- Add few drops of H2SO4 to stop the enzymatic reaction and adjust the PH to 2
- Filtrate the mix using a funnel fitted with filter paper
- Add butyl acetate solvent (1Vsolvant/2Vfiltrate)(4ml/8ml)
- Let rest for 2 minutes, then the organic phase is removed and the aqueous phase is discarded after decantation

(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



Crystallization:

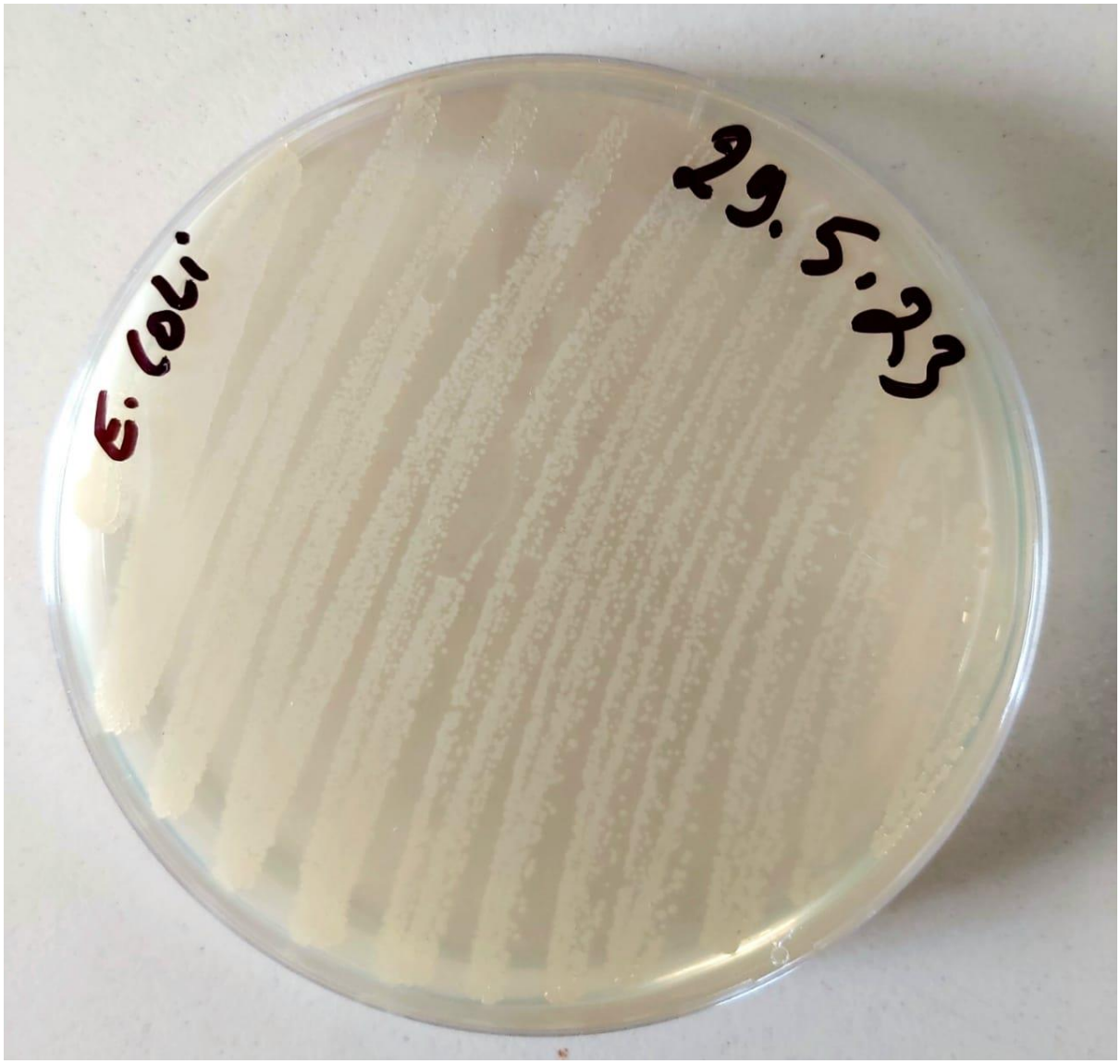
- Add 2% (W/V)(~0.1g/4.3ml) NaHCO3 to the aqueous phase medium
- Cool them at 4oC for about 7 days
- Filtrate them to harvest ampicillin sodium salt

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 simple on a disc
- Reverse the plate and incubate it at at 37o 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

The results show :

- no zone of inhibition in A1(sample taken before adjusting the 2)
- No zone of inhibition in the aqueous phaseA2 so the total produced ampicillin is extracted into the organic phaseA3
- 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 phaseB1 with an important one in the organic phaseB2 so PB isn't the right solvent for ampicillin extraction for crystallization.

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.

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
Tube1	1	9

20mg/ml		
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:

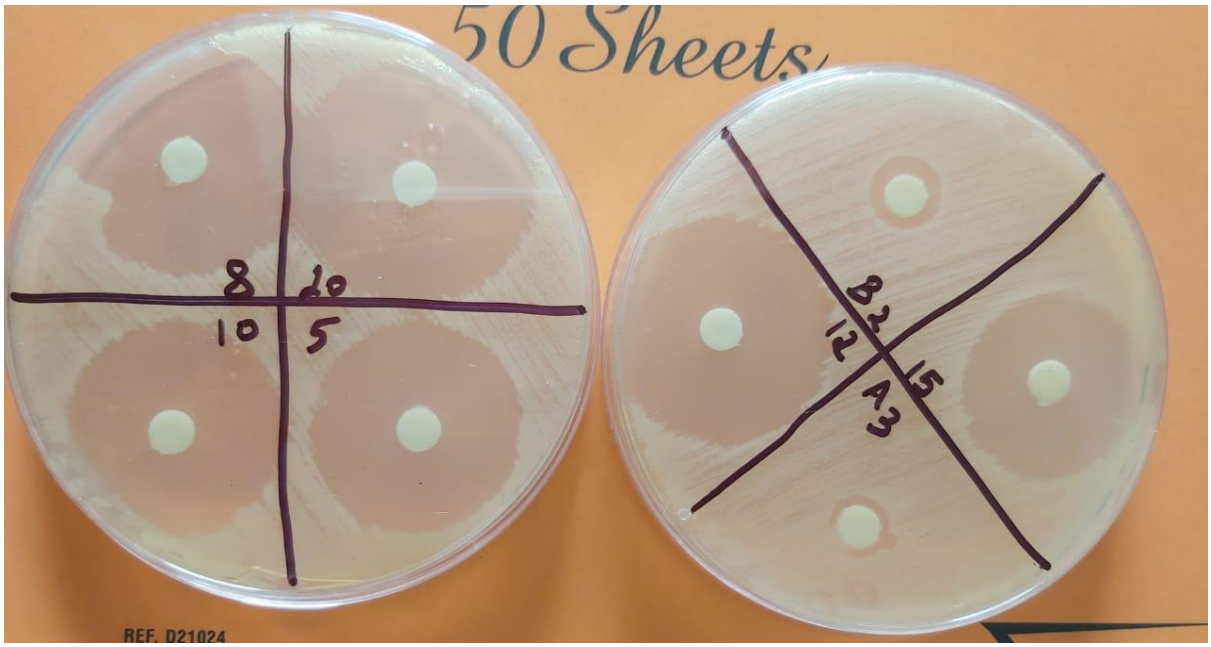
- Take 3 to 5 colonies of the isolated colonies with a loop, and we added them in 2ml sterile saline (NaCl 0.9%)
- Vortex the saline tube to create a smooth suspension.
- Adjust the turbidity of this suspension to a 0.5 McFarland standard(annex)
    - by adding more organism if the suspension is too 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

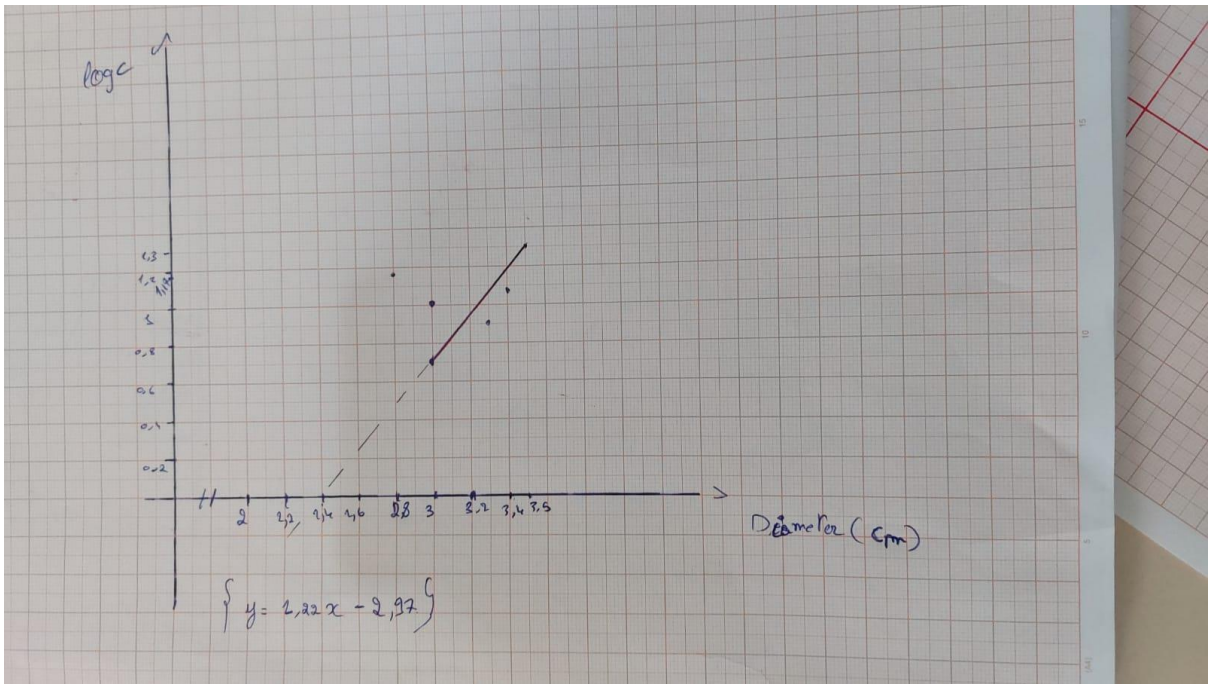
- After the growth time, measure the zone of inhibition that had appeared using a ruler.
- Draw a graph showing the concentration of amoxicillin as a function of the diameter in order to be able to quantify the produced ampicillin (diameter as a function of Log C).

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	?	?





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 1cm the concentration is 0.01 mg/ml

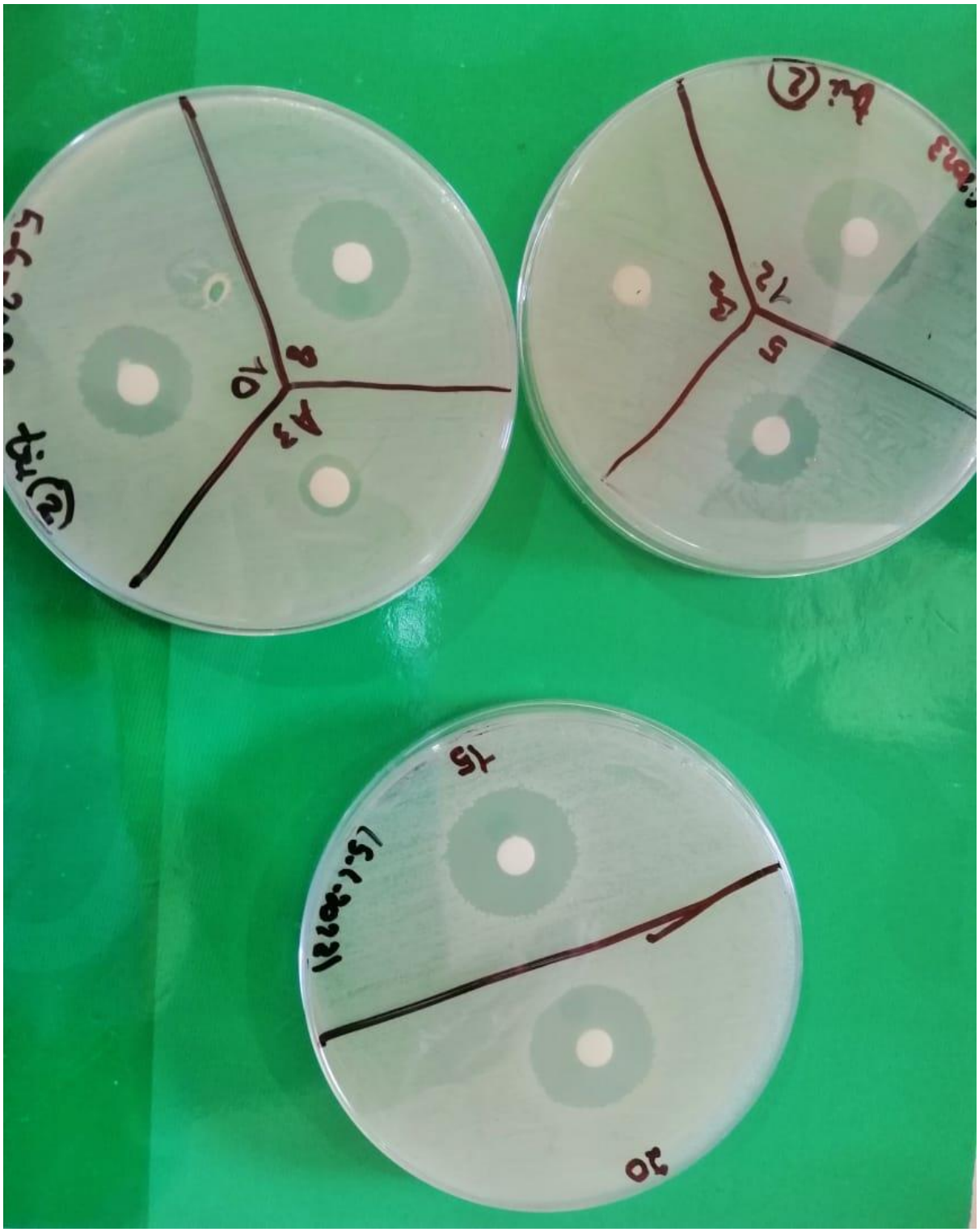
For an inhibition zone of 1.1 cm the concentration is 0.02 mg/ml

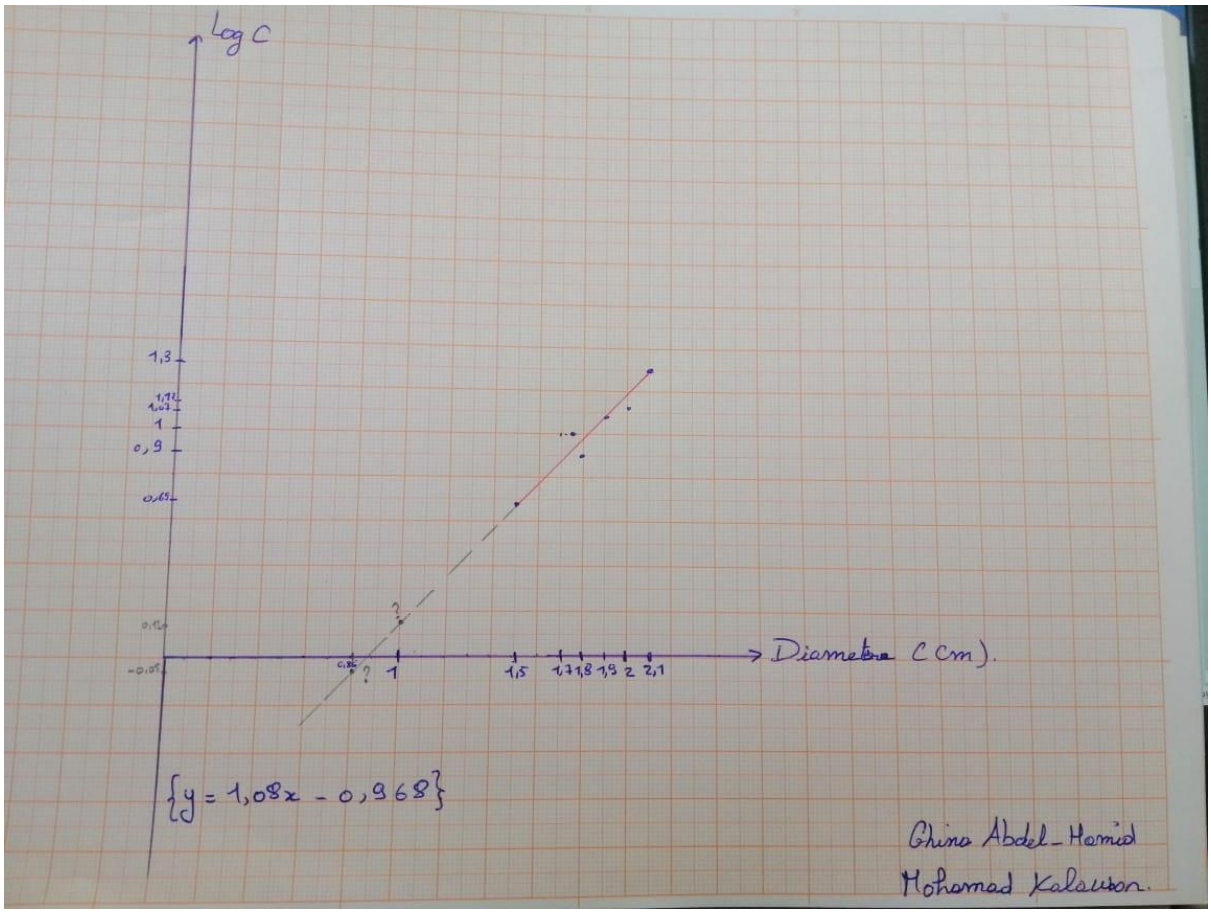
Results :

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	?	?





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 1cm 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

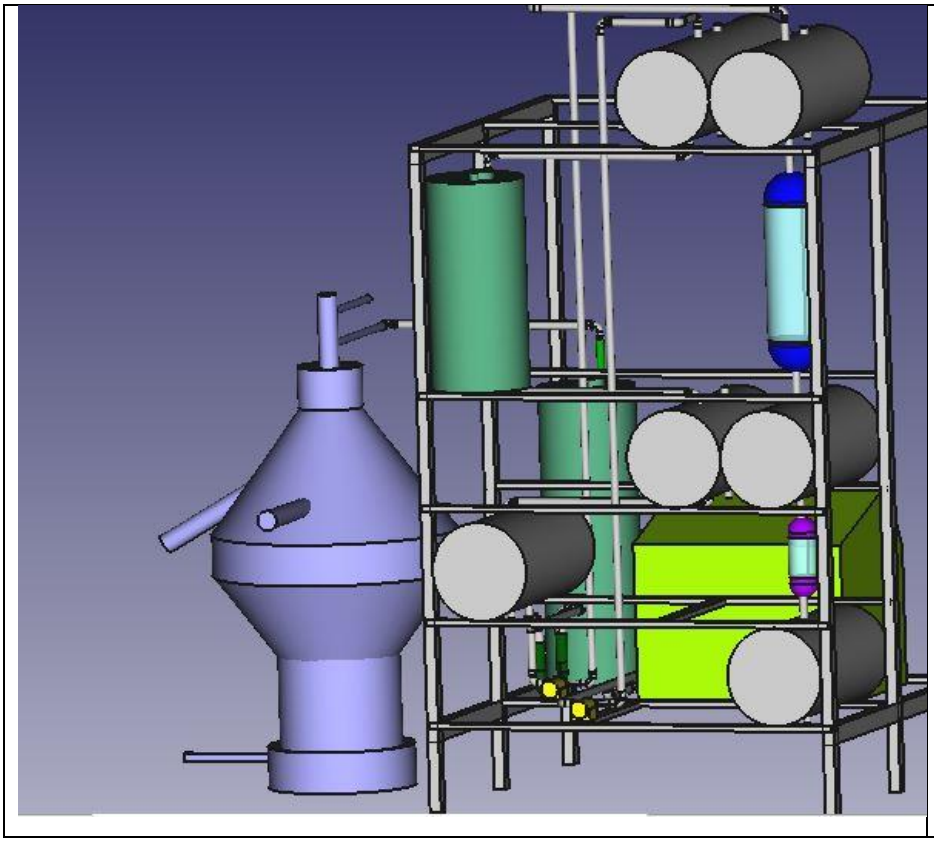
-Uncompleted inhibition in B2

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

16 Part IV: Generic Pilot Plant Design (Penicillin/Ampicillin/Aspirin)

16.1 Mechanical design

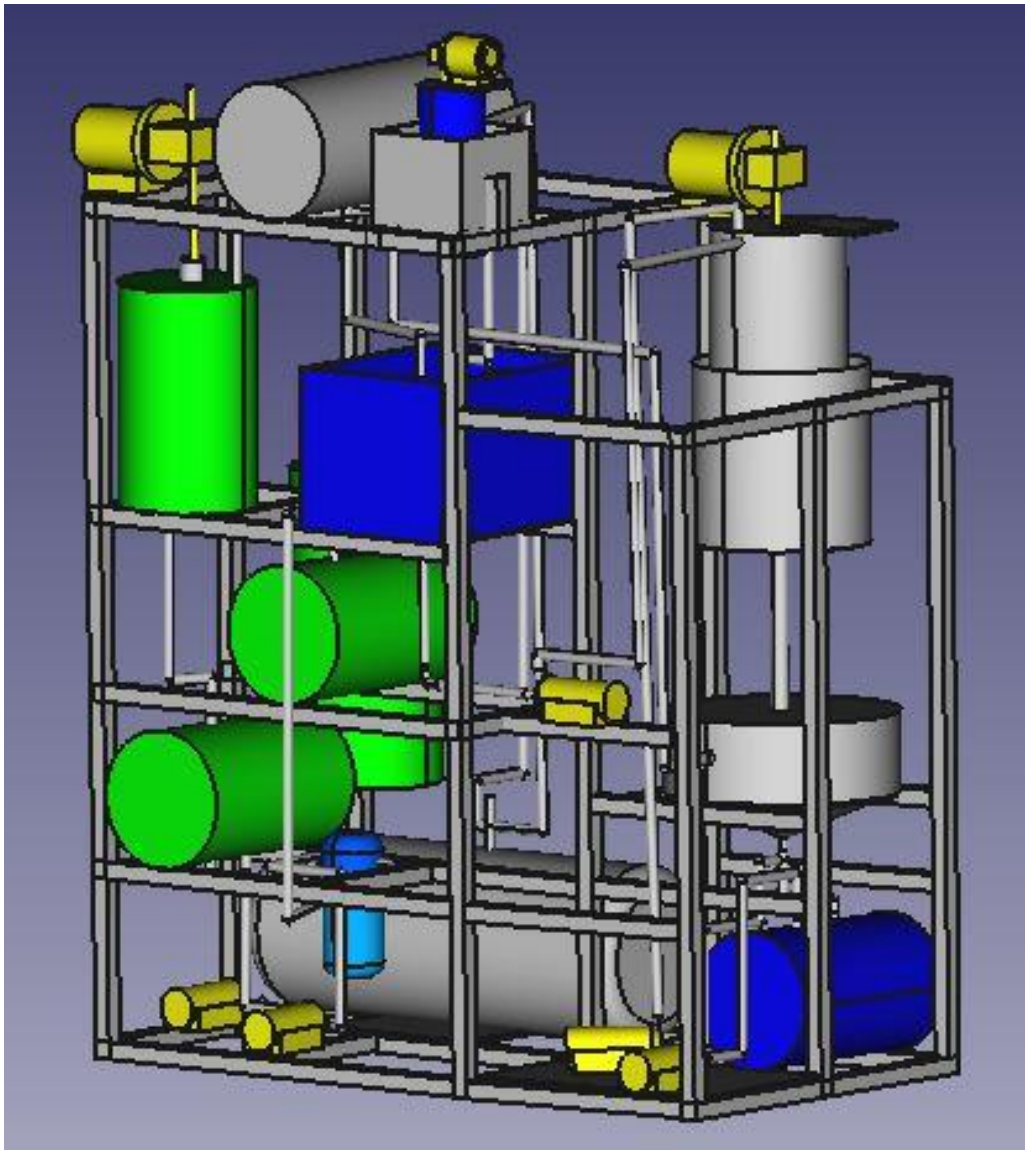
16.1.1 MEGBI penicillin/ampicillin/asprin pilot scale production old design



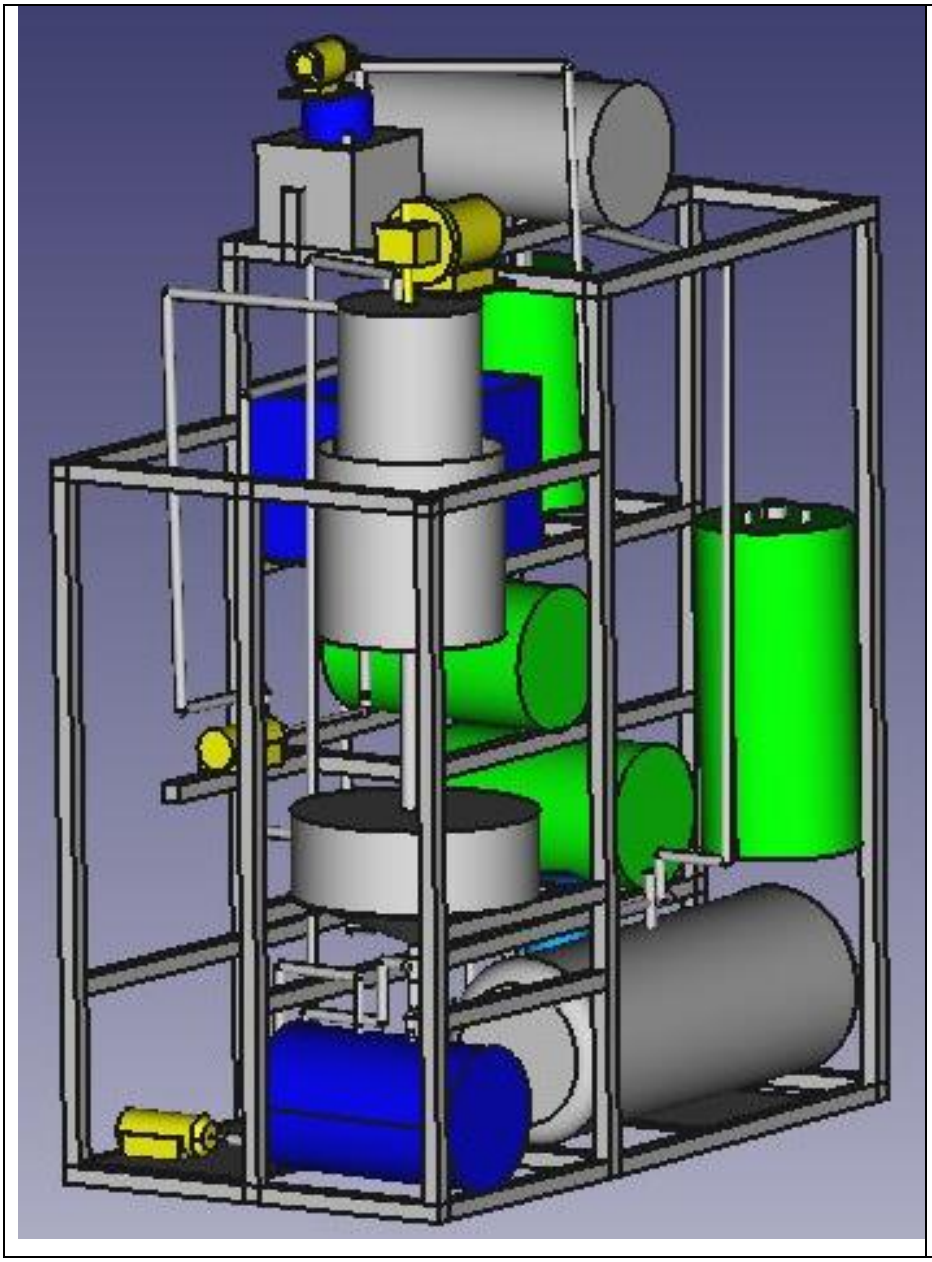
FreeCAD MEGBI pen/amp/asp pilot scale version 2015

060116MEGBI-VPP_Assembly_simplified_ma

16.1.2 update for pilot plant ASPIRIN/PENICILLIN production 18/11/2023



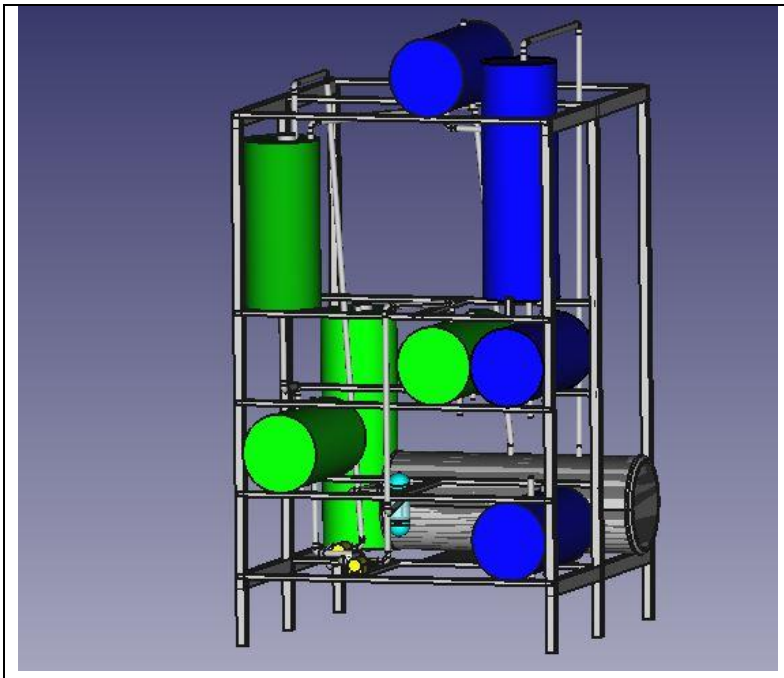
16.1.3 update for pilot plant ASPIRIN/PENICILLIN production 23/11/2023



FreeCAD design ASPIRIN/PENICILLIN Pilot plant 23/11/2023

aspirin filtration new design0.FCStd

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



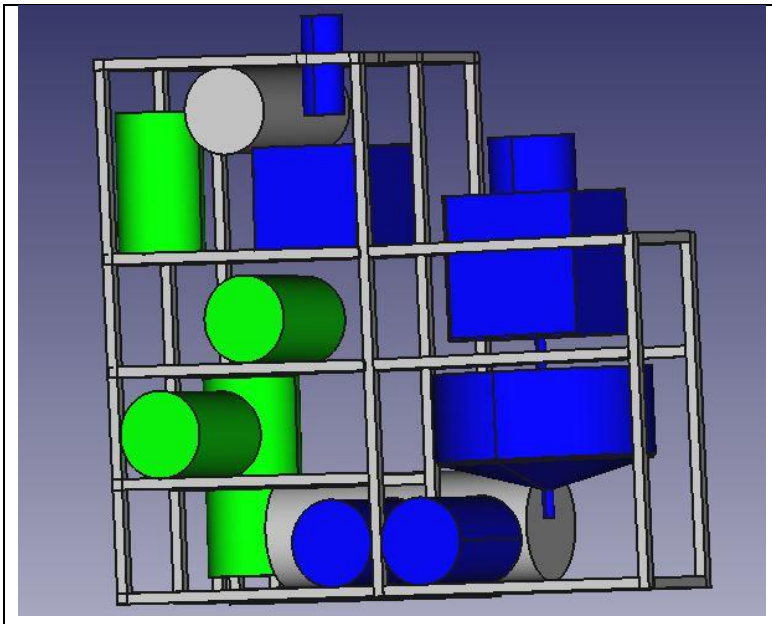
Blue tanks : Aspirin, Green tanks : Penicillin/Ampicillin

FreeCAD design MEGBI Pen/Amp/Asp pilot scale version

FreeCAD Pilot Scale New design.FCStd

28/11/2022

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



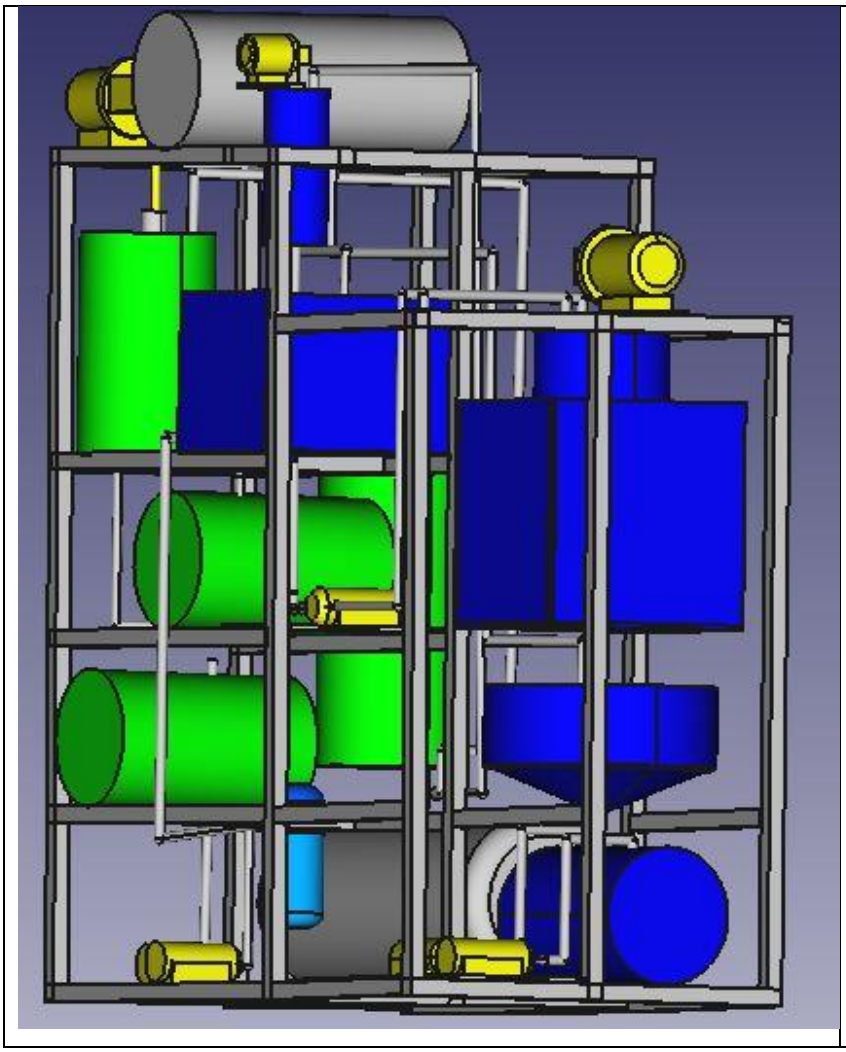
Blue tanks : Aspirin, Green tanks : Penicillin/Ampicillin

FreeCAD design Pen/Amp/Asp pilot scale version

aspirin filtration.FCStd

16/12/2022

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



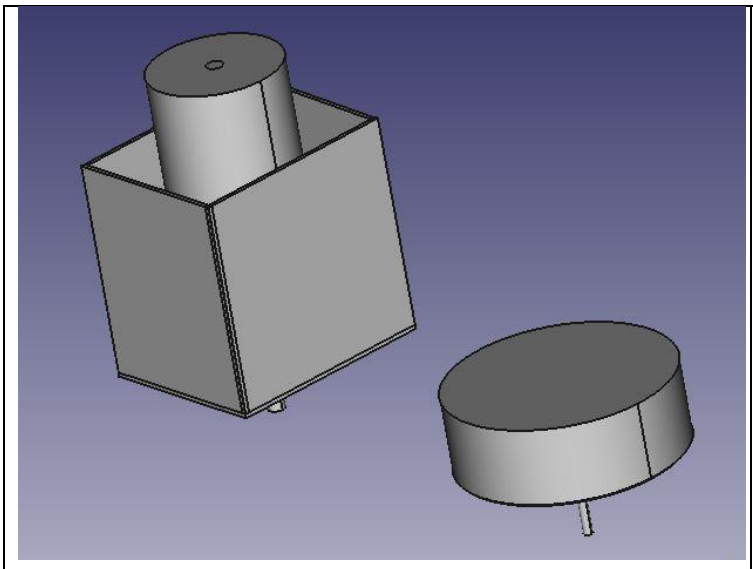
Blue tanks: Aspirin,

Green tanks: Penicillin/ Ampicillin

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

aspirin filtration new design.FCStd

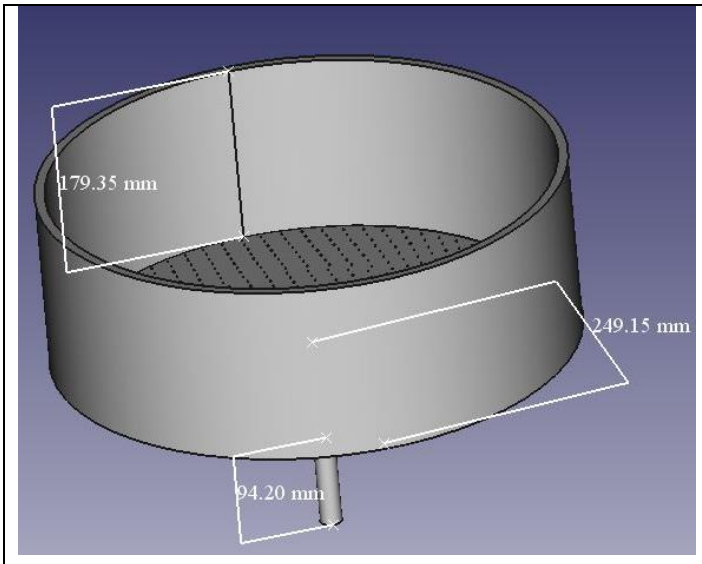
16.1.7 MEGBI Aspirin filter and crystallizer pilot scale FreeCAD design 09/01/2023

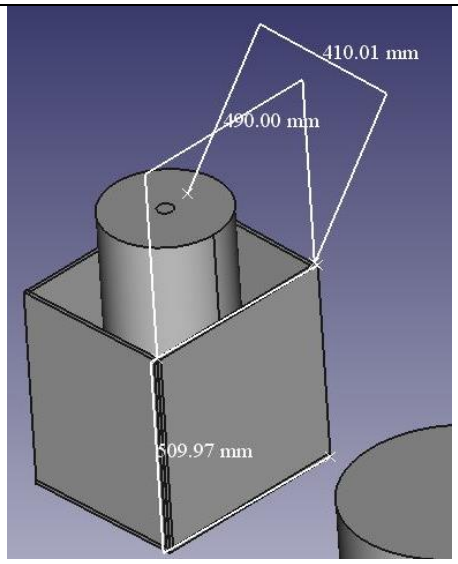


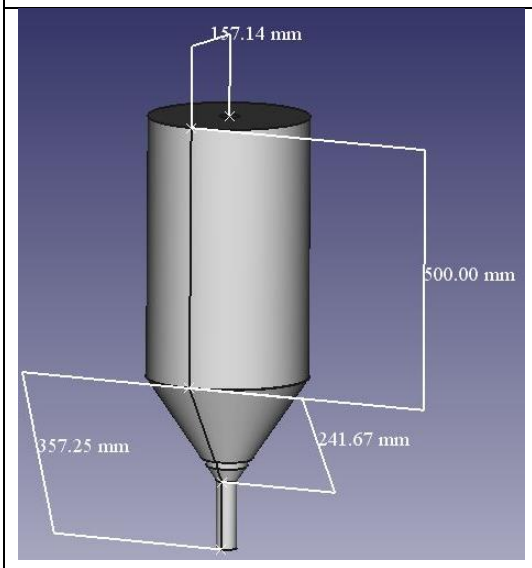
FreeCAD design aspirin filter/crystallizer pilot scale version 10/01/2023

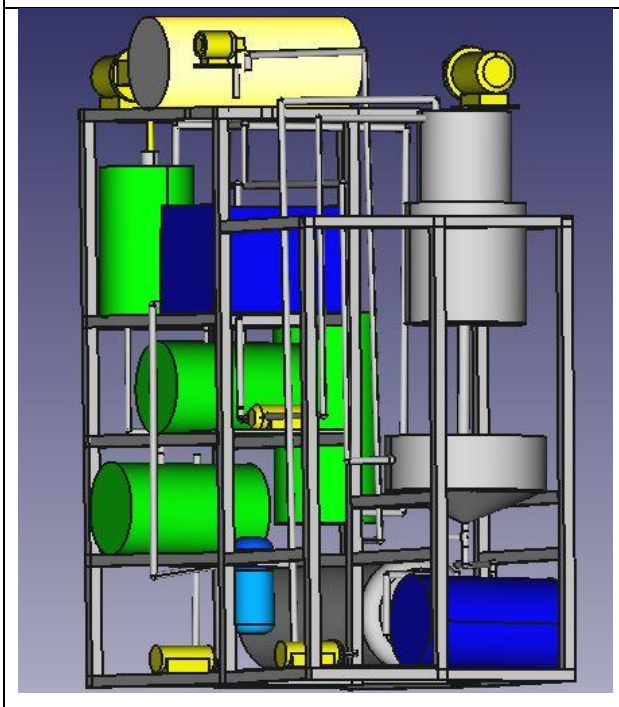
aspirin filter + crystallizer.FCStd

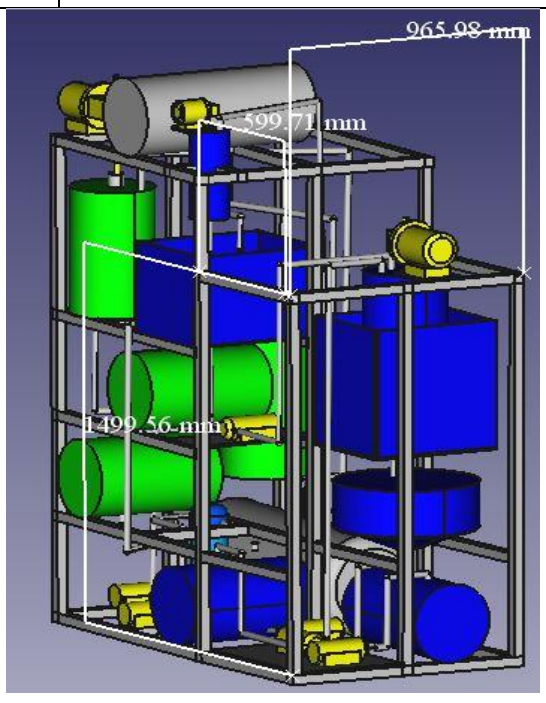
16.1.8 Sizes of filter/crystallizer aspirin pilot plan











17 Part V: Aspirin Pilot Plant

17.1 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 :

- 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 acccording to the mechanical design.

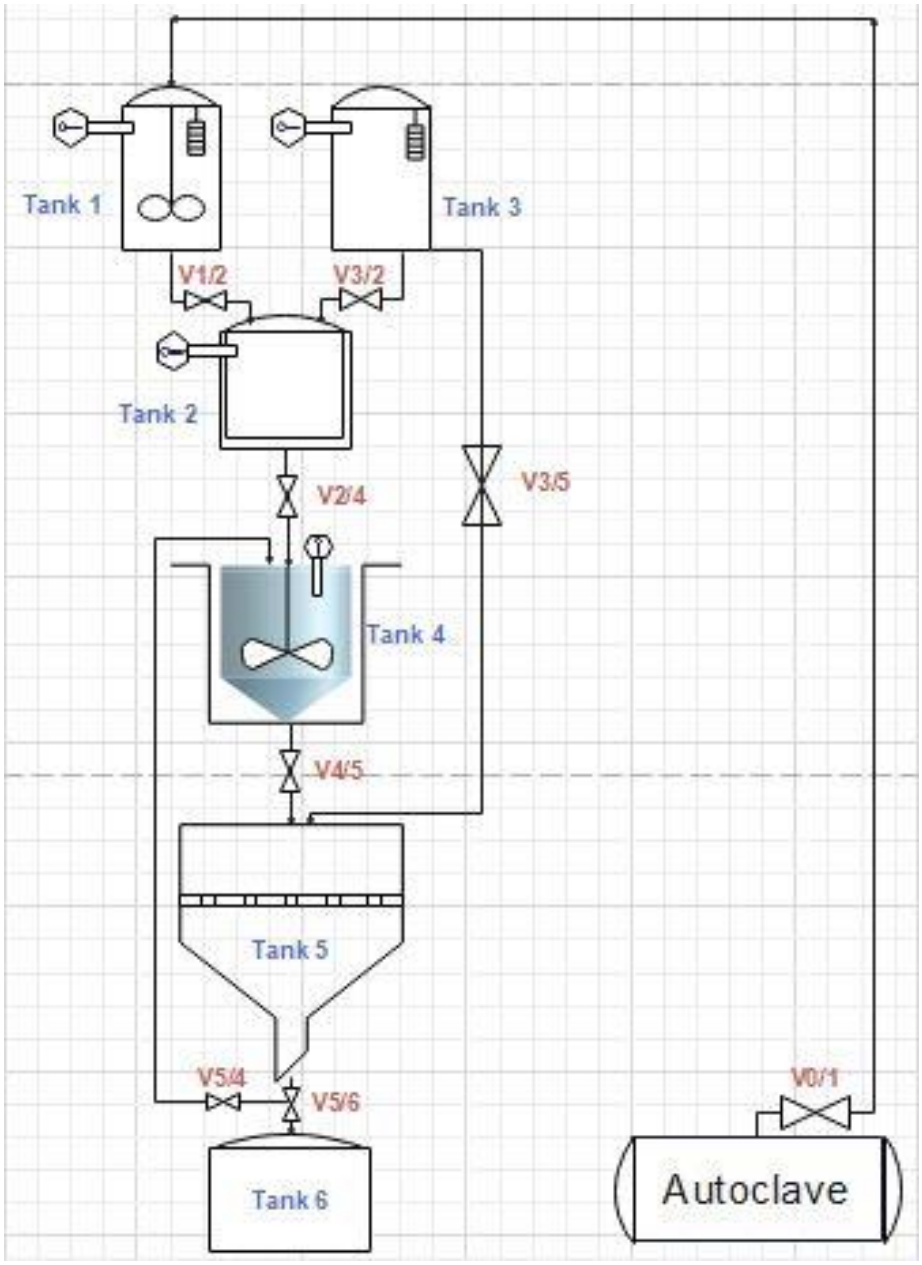
Safety requirements :

- The system shall be enclosed completely to ensure no toxic gas and to avoid contact with the burns from concentrated acid (H_2SO_4).

- The system shall be enclosed during the sterilization step to avoid the burns form hot vapor used to sterilization.

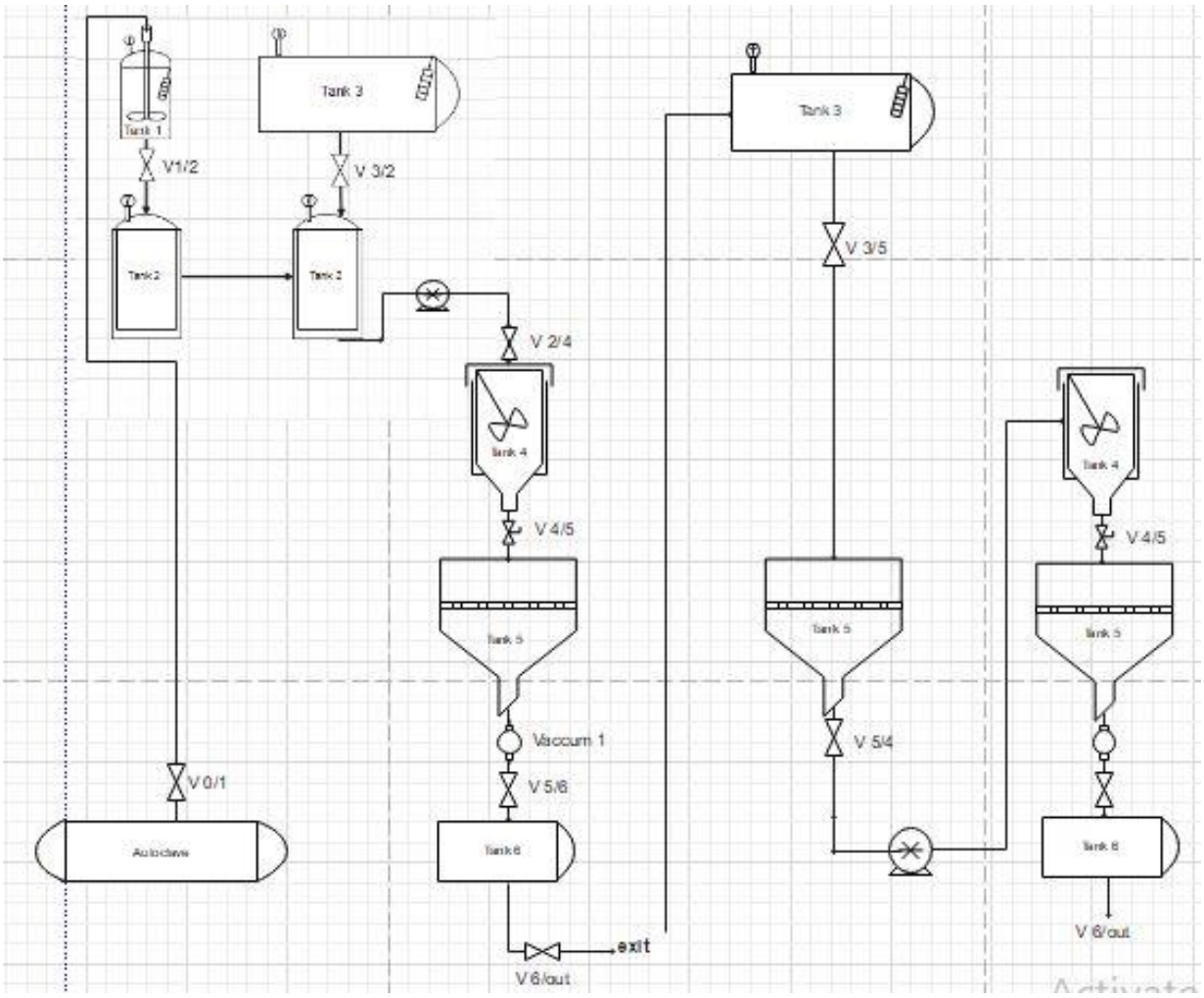
17.2 Pilot plant system design

17.2.1 Dynamic View of Aspirin Pilot Plant Production



02/18/2023 Word file contain the E-draw file of Appoximate View for aspirin pilot plant

word approxmative view 18.02.23.docx



02/18/2023 word file contain the E-draw file of Dynamic view for aspirin pilot plant

Word dynamic view 18.2.23.docx

17.2.1.1 Operation Sequence

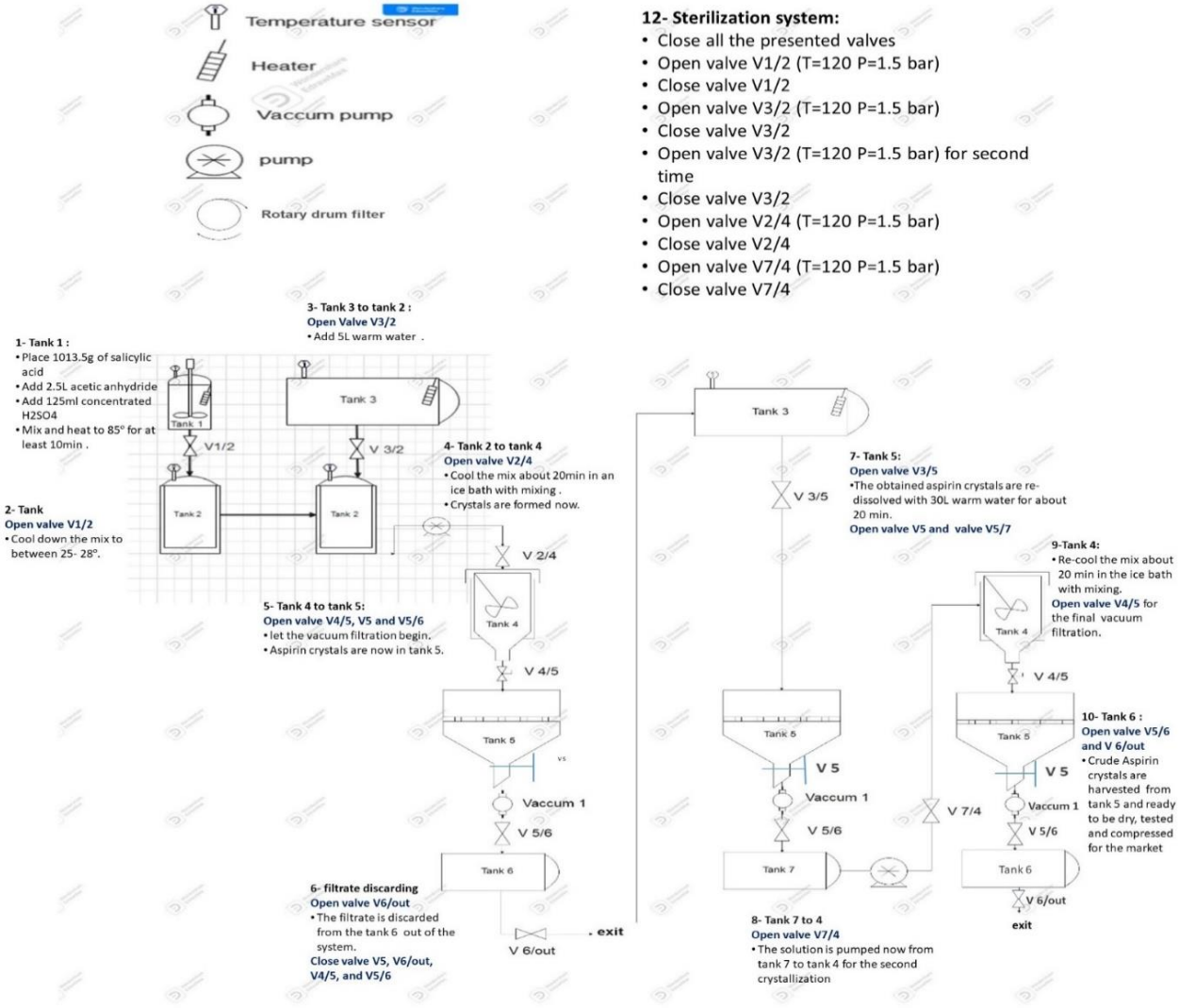
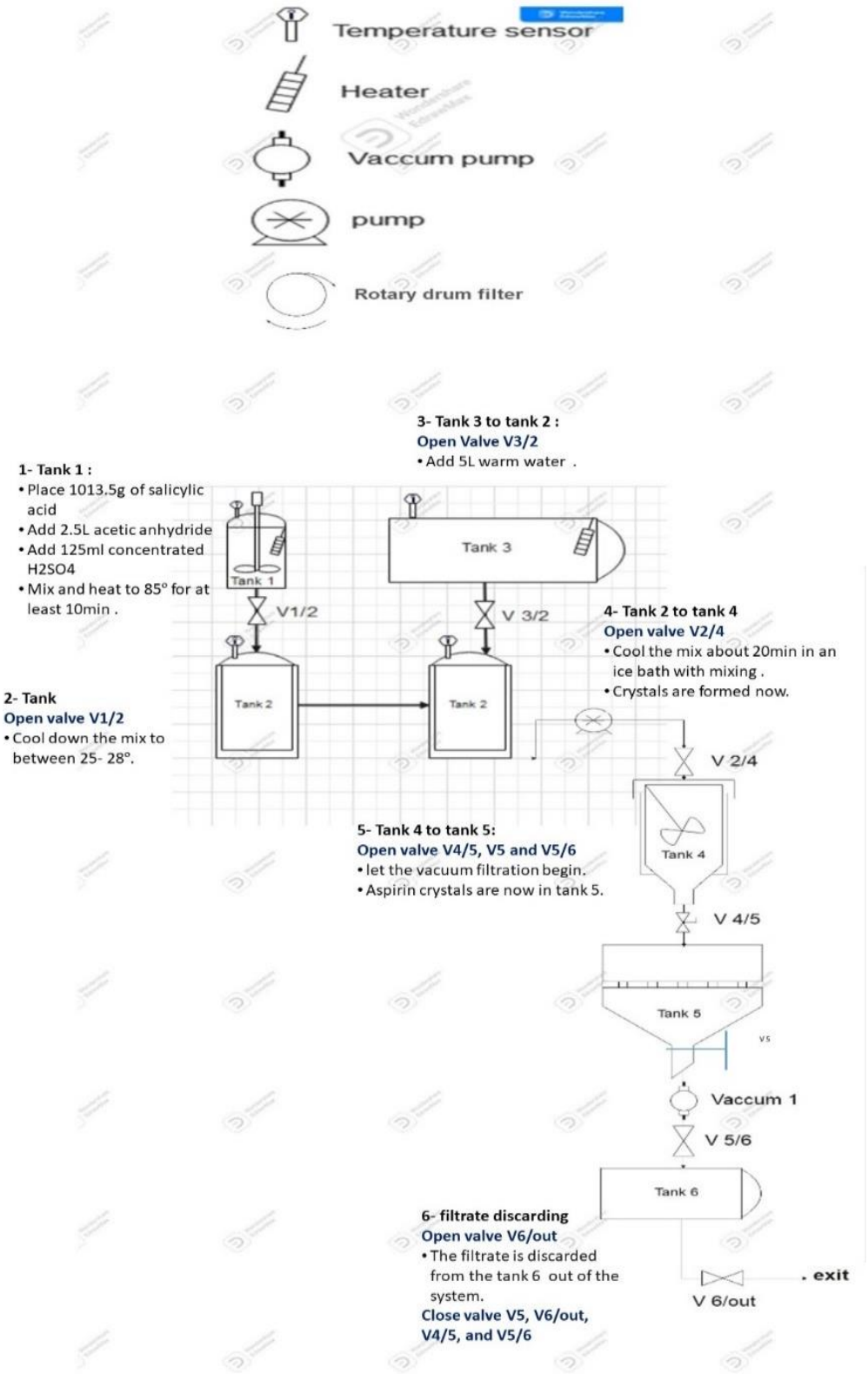


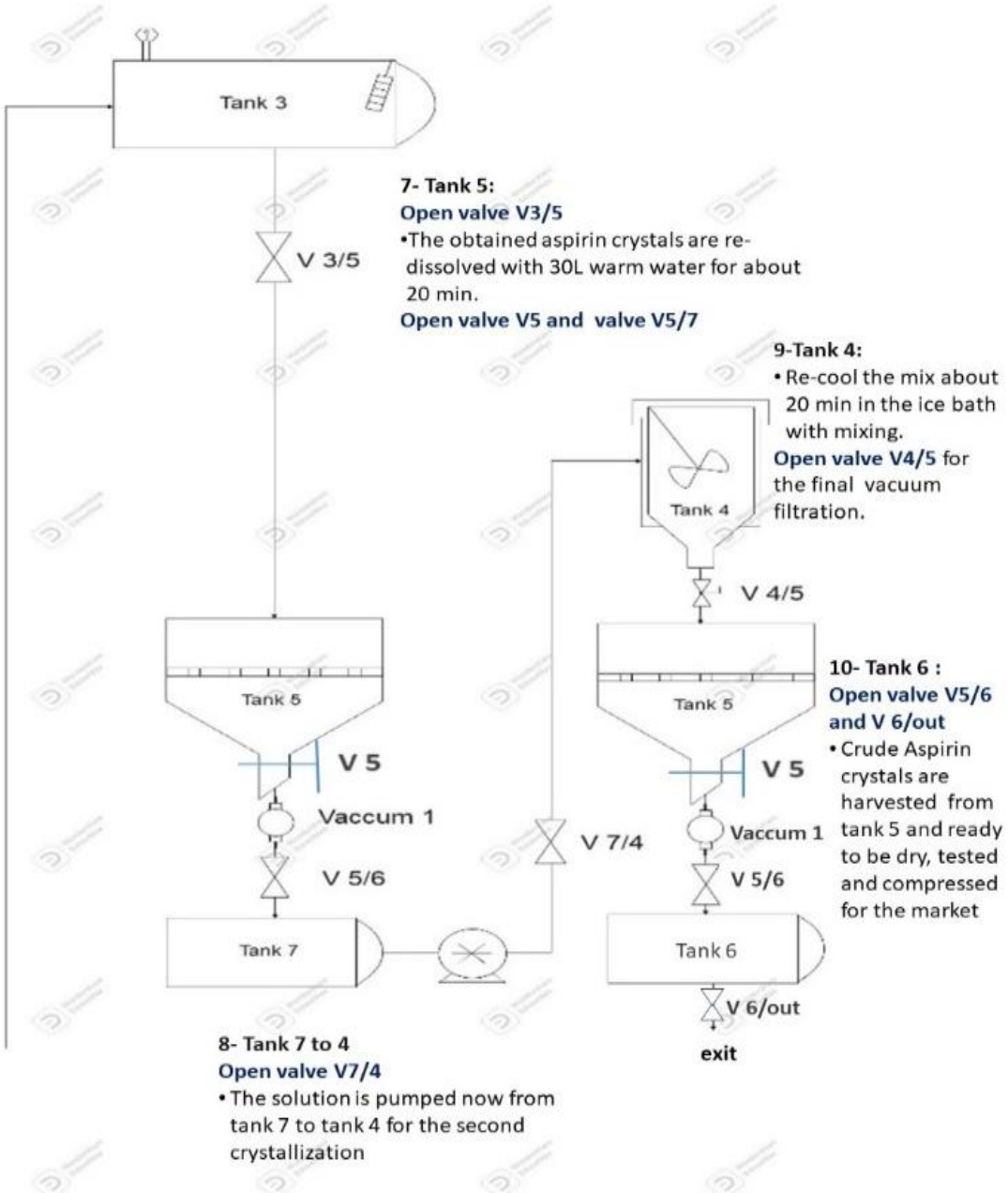
Figure 1 : Dynamic view of pilot plant aspirin production system

Zoomed View:


Temperature sensor
Heater
Vaccum pump
pump
Rotary drum filter
1- Tank 1 :
• Place 1013.5g of salicylic acid
• Add 2.5L acetic anhydride
• Add 125ml concentrated H2SO4
• Mix and heat to 85° for at least 10min .
2- Tank
Open valve V1/2
• Cool down the mix to between 25- 28°.
3- Tank 3 to tank 2 :
Open Valve V3/2
• Add 5L warm water .
Tank 1
V1/2
Tank 2
Tank 3
V 3/2
Tank 2
4- Tank 2 to tank 4
Open valve V2/4
• Cool the mix about 20min in an ice bath with mixing .
• Crystals are formed now.
V 2/4
Tank 4
5- Tank 4 to tank 5:
Open valve V4/5, V5 and V5/6
• let the vacuum filtration begin.
• Aspirin crystals are now in tank 5.
V 4/5
Tank 5
V5
Vaccum 1
V 5/6
Tank 6
6- filtrate discarding
Open valve V6/out
• The filtrate is discarded from the tank 6 out of the system.
Close valve V5, V6/out, V4/5, and V5/6
. exit
V 6/out

12- Sterilization system:

- Close all the presented valves
- Open valve V1/2 (T=120 P=1.5 bar)
- Close valve V1/2
- Open valve V3/2 (T=120 P=1.5 bar)
- Close valve V3/2
- Open valve V3/2 (T=120 P=1.5 bar) for second time
- Close valve V3/2
- Open valve V2/4 (T=120 P=1.5 bar)
- Close valve V2/4
- Open valve V7/4 (T=120 P=1.5 bar)
- Close valve V7/4



7- Tank 5:
Open valve V3/5
•The obtained aspirin crystals are re-dissolved with 30L warm water for about 20 min.
Open valve V5 and valve V5/7

9-Tank 4:
• Re-cool the mix about 20 min in the ice bath with mixing.
Open valve V4/5 for the final vacuum filtration.

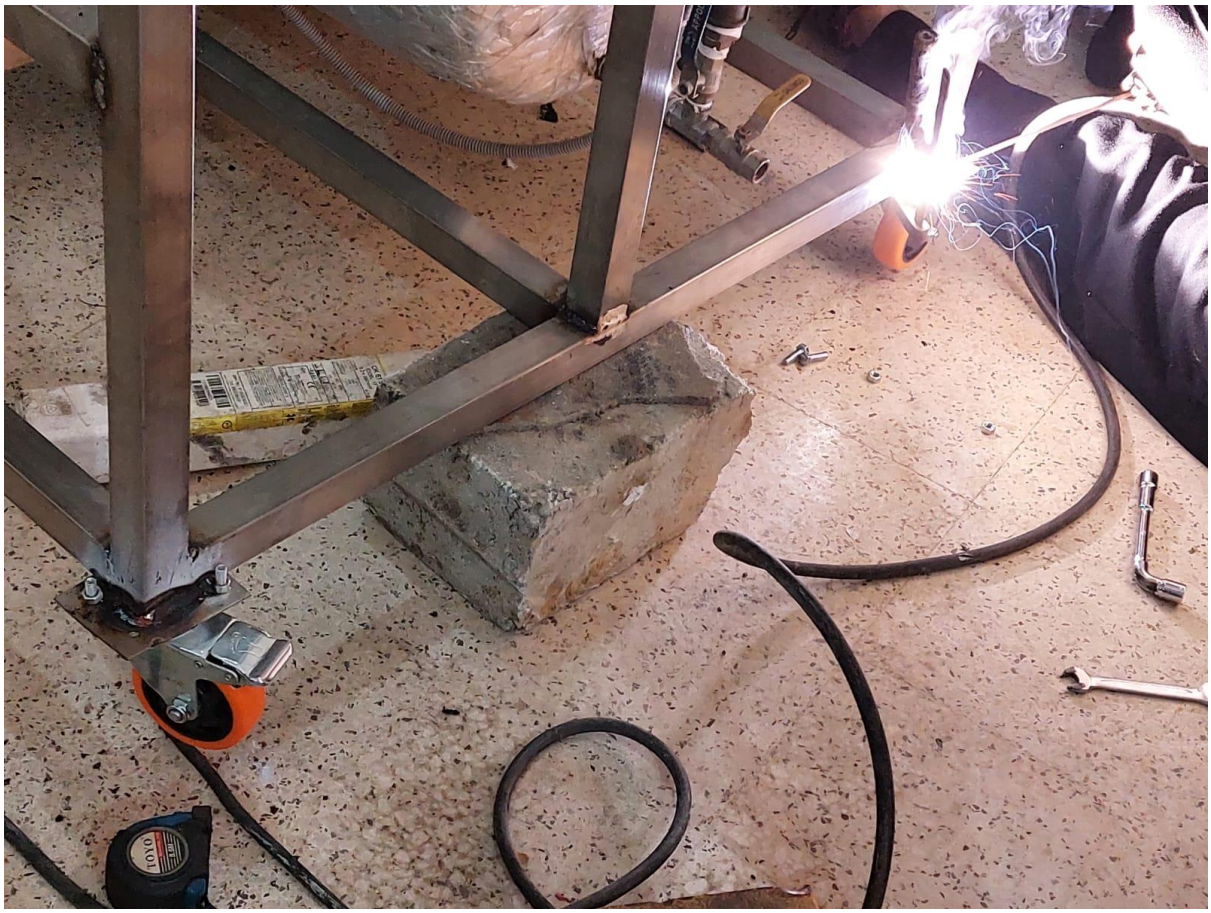
10- Tank 6 :
Open valve V5/6 and V 6/out
• Crude Aspirin crystals are harvested from tank 5 and ready to be dry, tested and compressed for the market

8- Tank 7 to 4
Open valve V7/4
• The solution is pumped now from tank 7 to tank 4 for the second crystallization

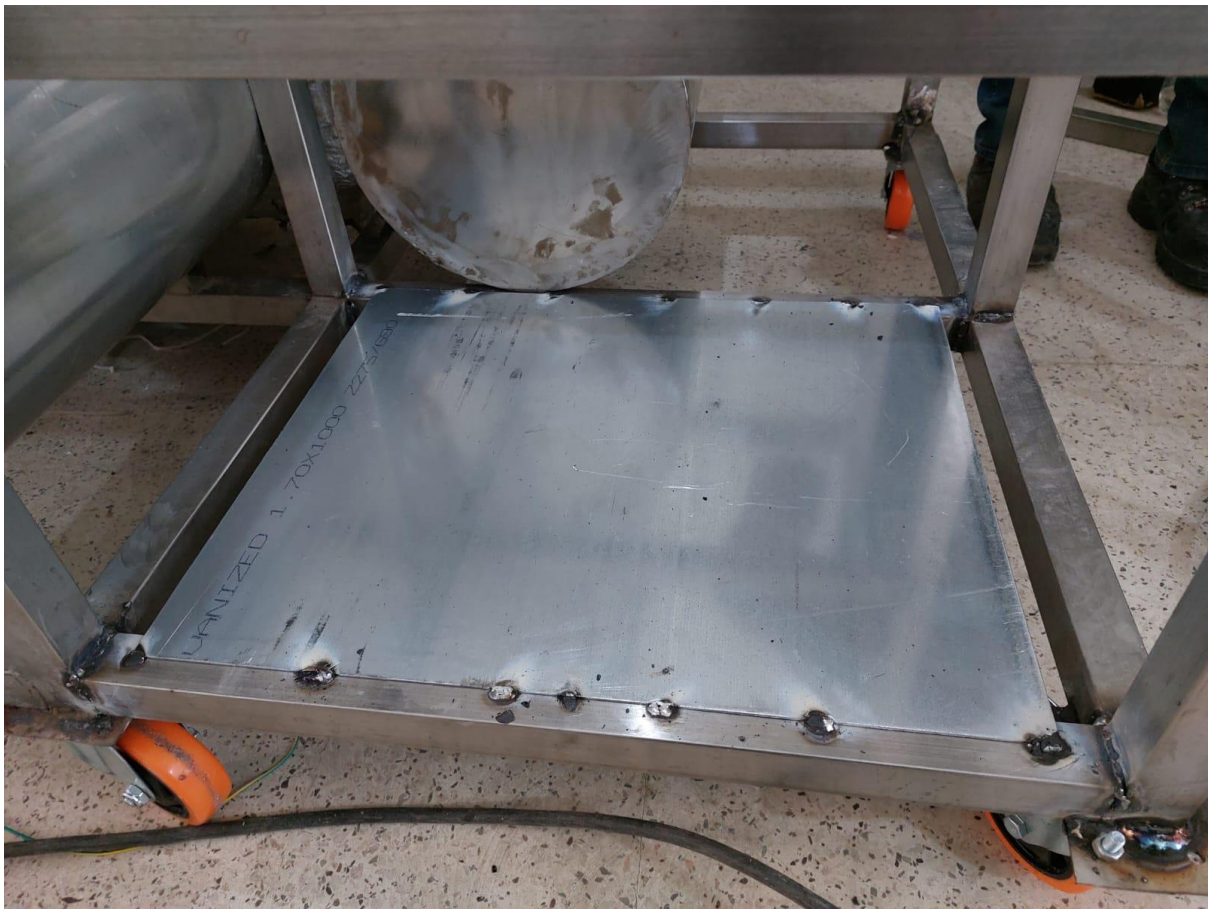
17.3 Aspirin pilot plant Mechanical Realization

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

17.3.1.1 Wheel stand installation :(19/01/2023)




SALEMCO


UNIZED 1.70X1000 2275/SN

Autoclave place editing :(20/01/2023)


SALEMCO

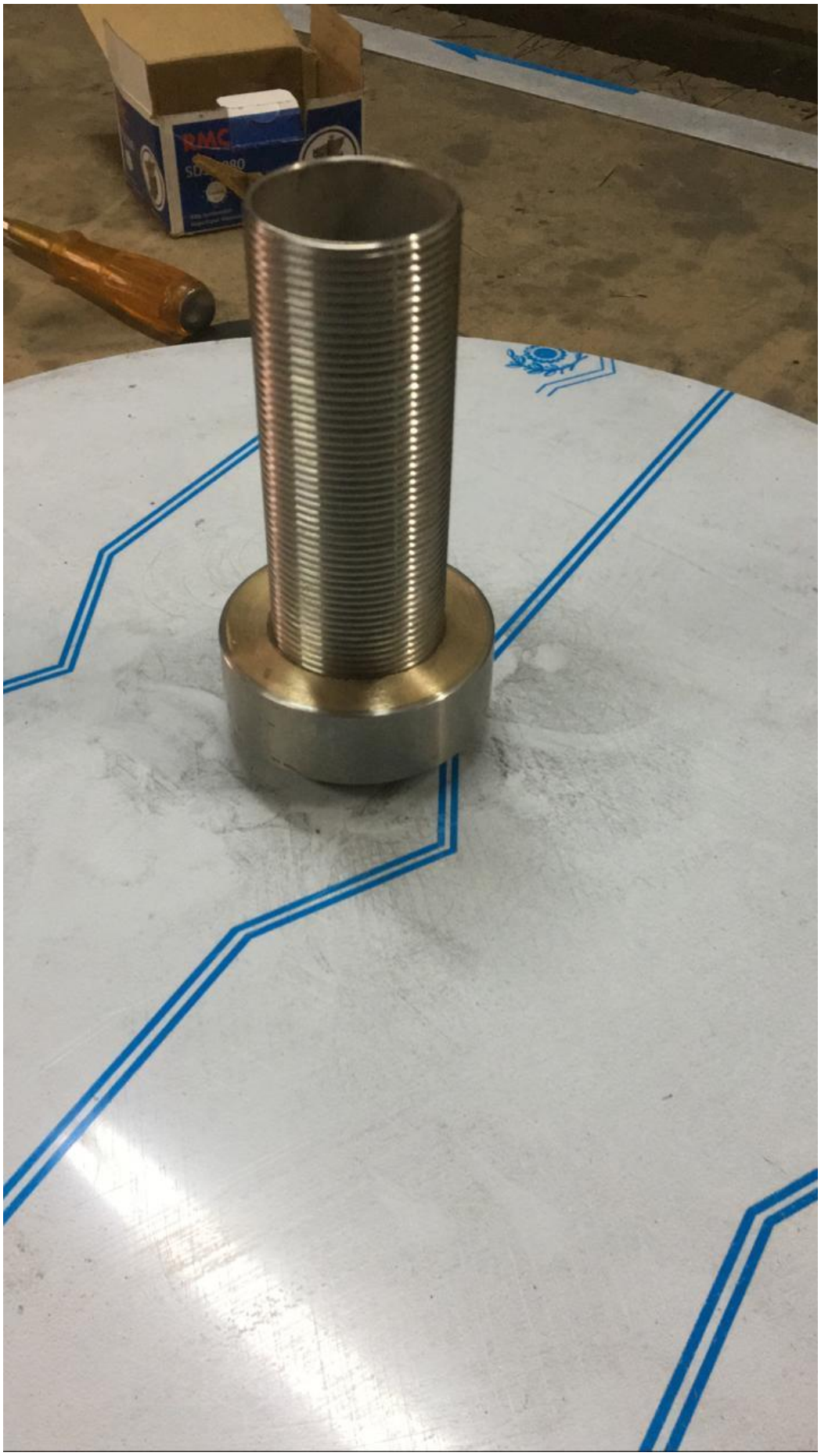
Bath for tank 2 (cooler) + tank 3 (crystallizer) and testing :(22/01/2023)-(04/07/2023)

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

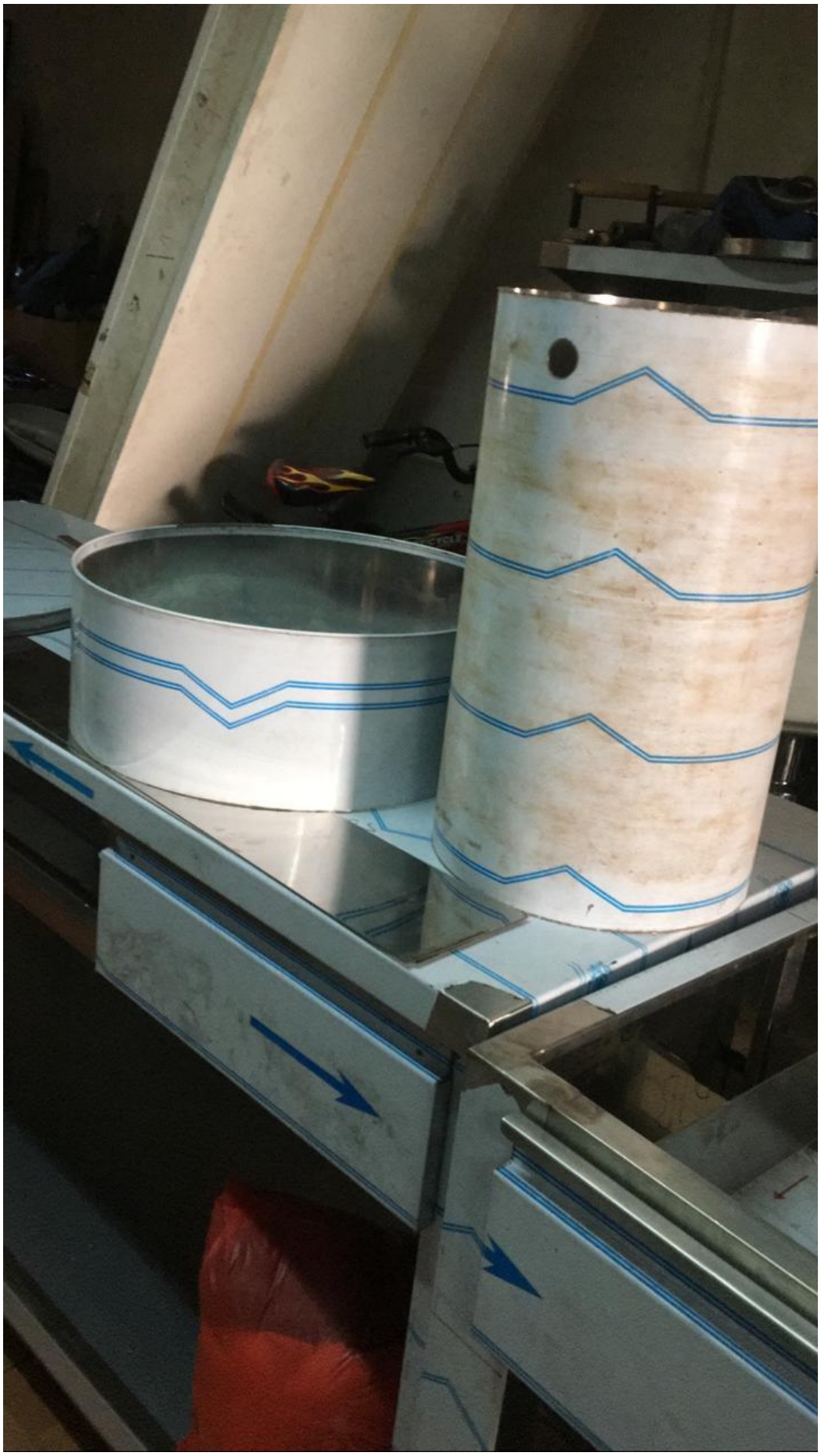

A. B. S.
A. B. S.
ALIDAD
Pilot 1929


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

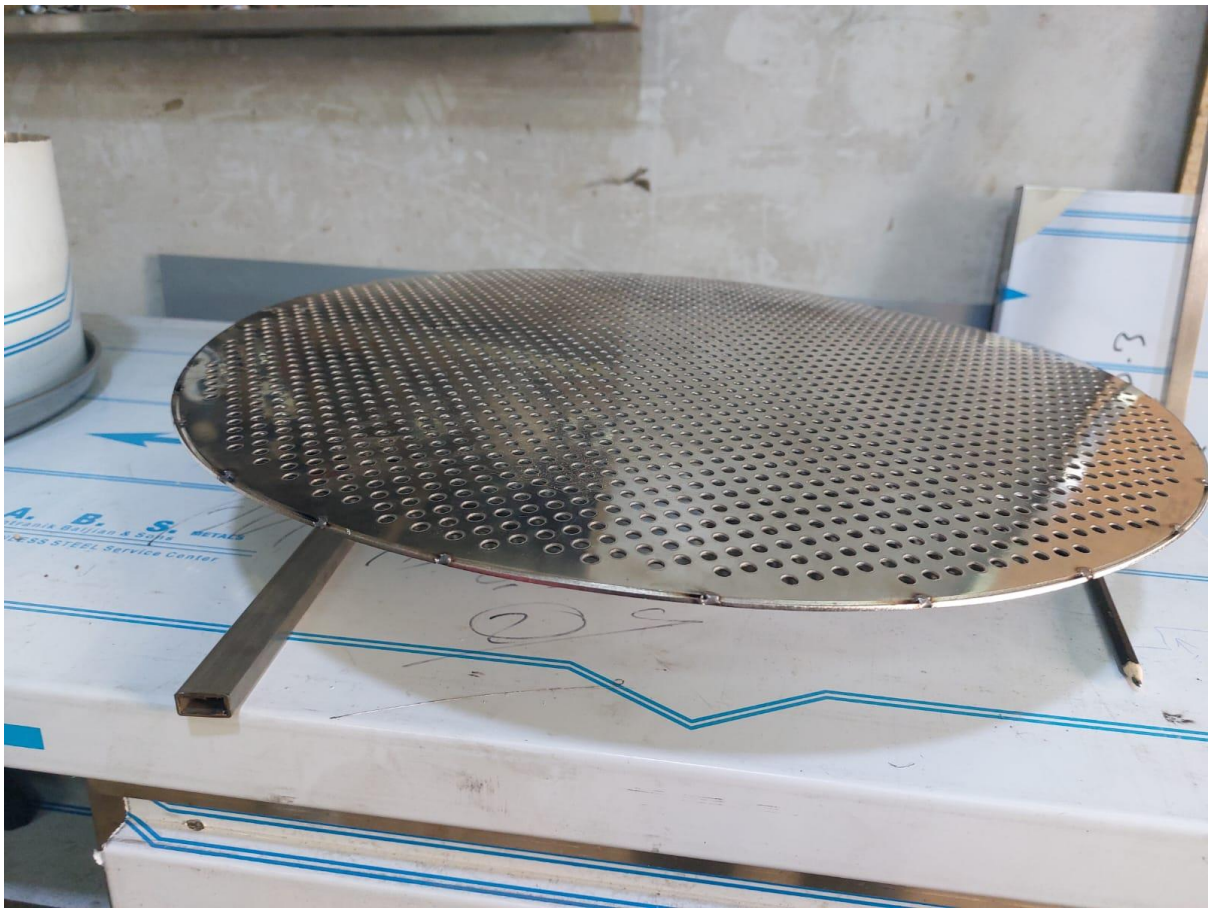
Cover for vacuum filter :(09/03/2023)


RMC

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



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



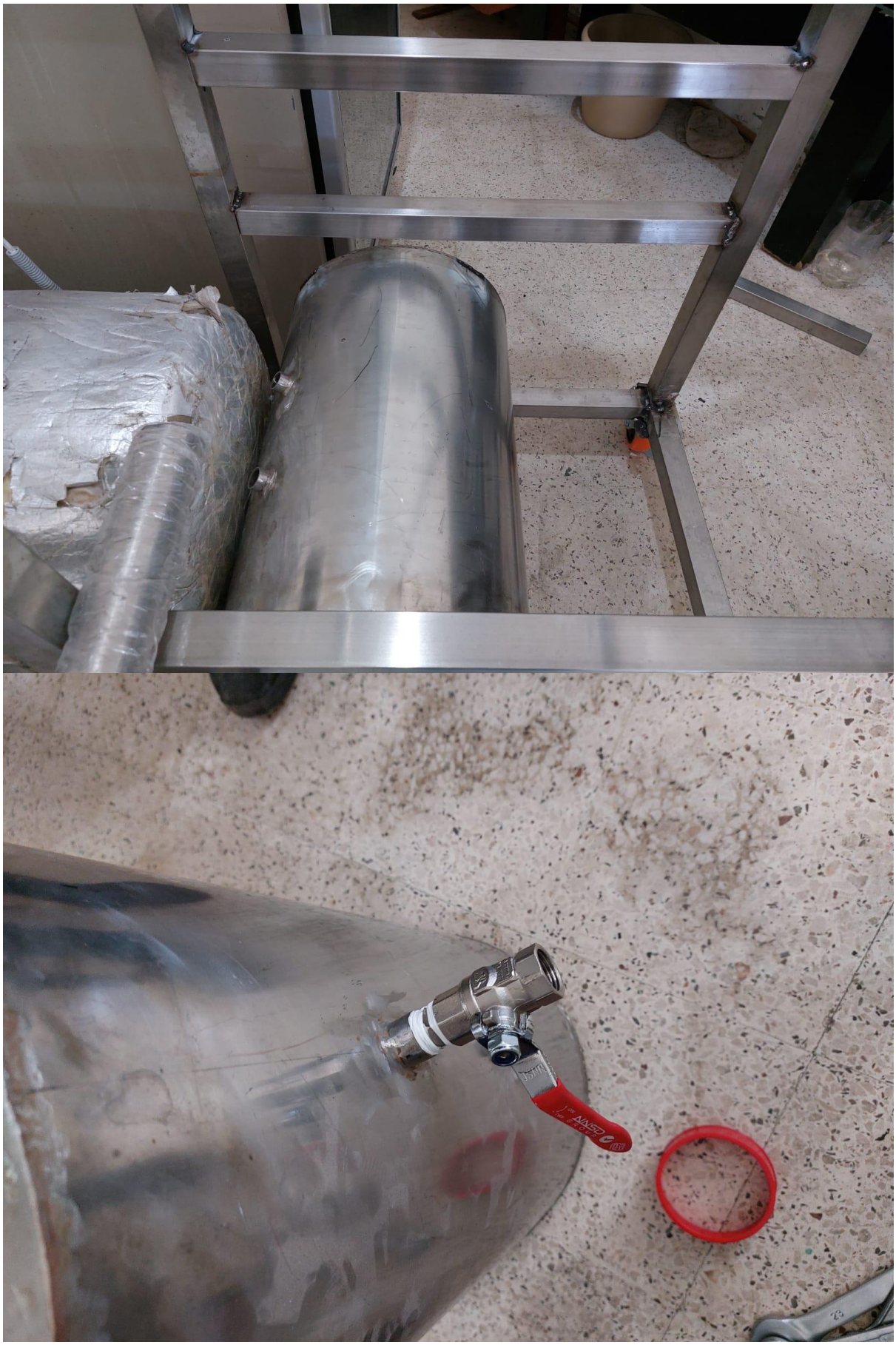
Vacuum filter realisation :(17/03/2023)


MITECH
A. B. S. METALS
Antranik Baljian & Sons
1AL1

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

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

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



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

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

Reactor for aspirin:(20/10/2023)

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

17.4 Test specification

17.4.1 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

17.4.2 Safety precaution

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

17.4.3 Autoclave operation sequence

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°C / P= 20000 Pa), operate the autoclave valve system to open

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

17.4.4 Autoclave System testcases

17.4.4.1 001: test the resistor of the autoclave

Step	Step Description	Expected Result
Precondition	System is off	
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	

17.4.4.2 002: 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 th 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 temperatur 121 °C
Open the manual discharge valve	Open the discharge valve manually	Pressure should decrease the steam vent off the syste
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	

17.4.5 Aspirin Production System Test Specification

17.4.5.1 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.

17.4.5.2 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.

17.4.5.3 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).

17.4.6 Aspirin Production operation sequence

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

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.

17.4.7 Aspirin Production System testcases

17.4.7.1 003: 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	

17.4.7.2 004: 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) (crystallizer---->crystallizer valve V3)	The mixture transferred to the filter (tank 5)
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
Postcondition	system is OFF	

17.5 System tests

17.5.1 003: 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

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

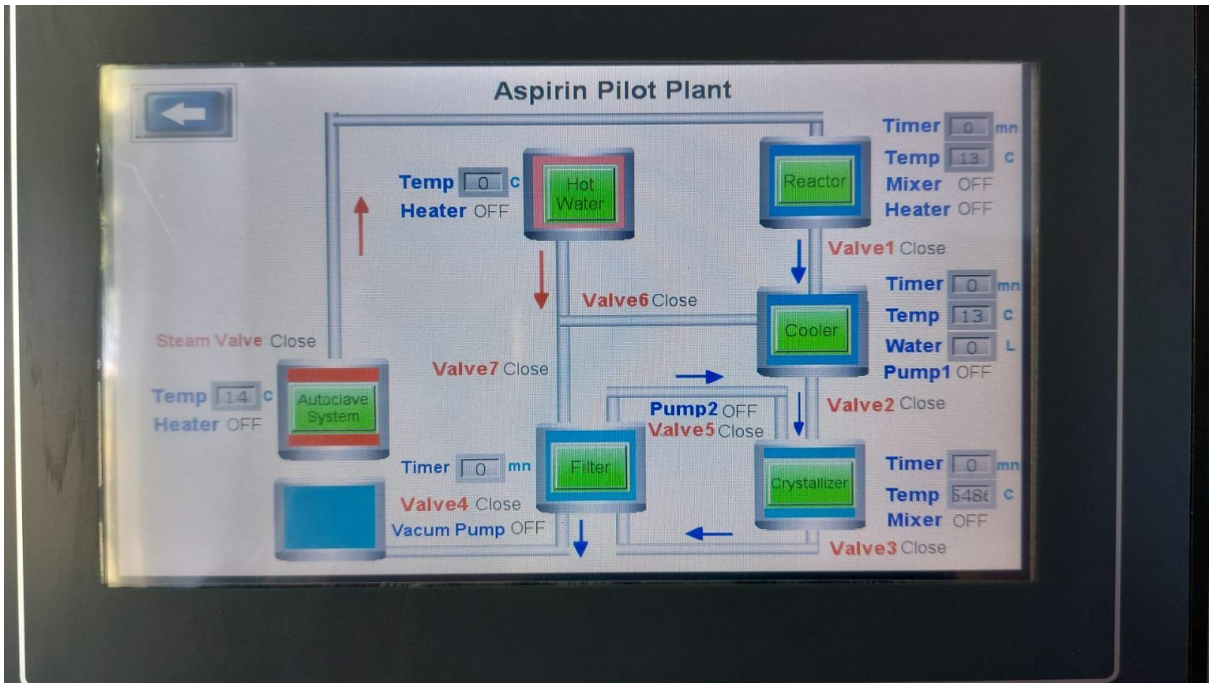
in the next test we will fix all those problems and try another test with water only.

Video003 (1): Testing Valves and mixers (water test 1) 28/11/2023

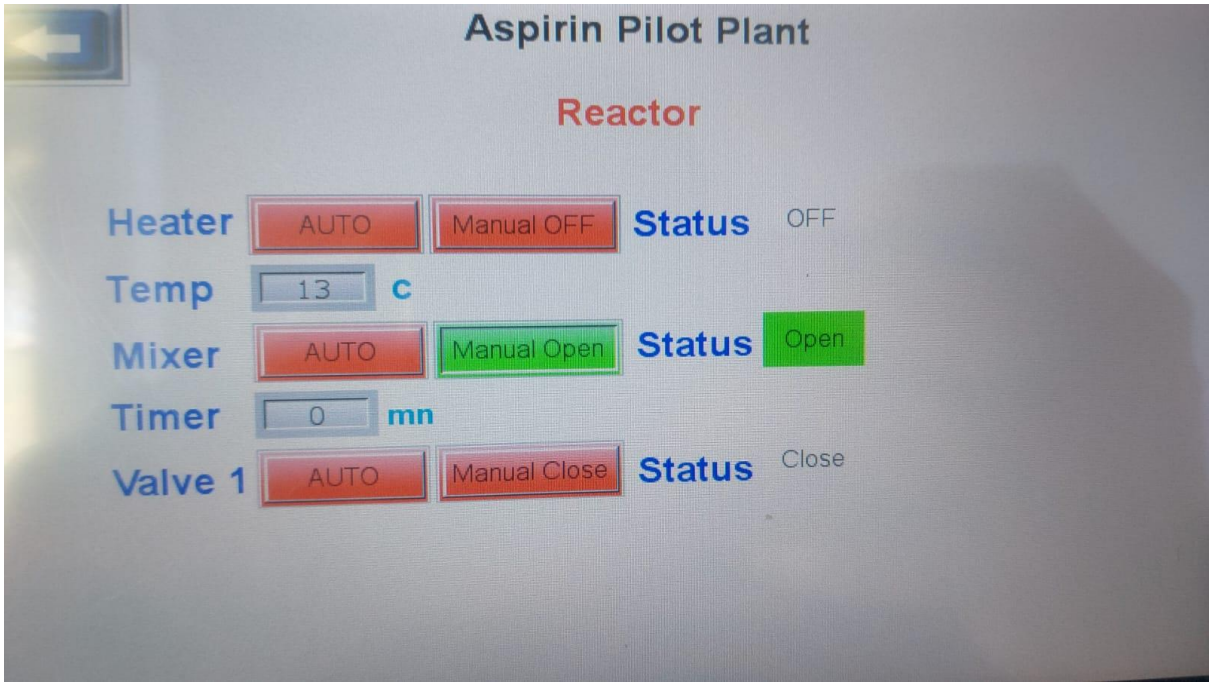
WhatsApp Video 2023-11-30 at 9.36.27

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

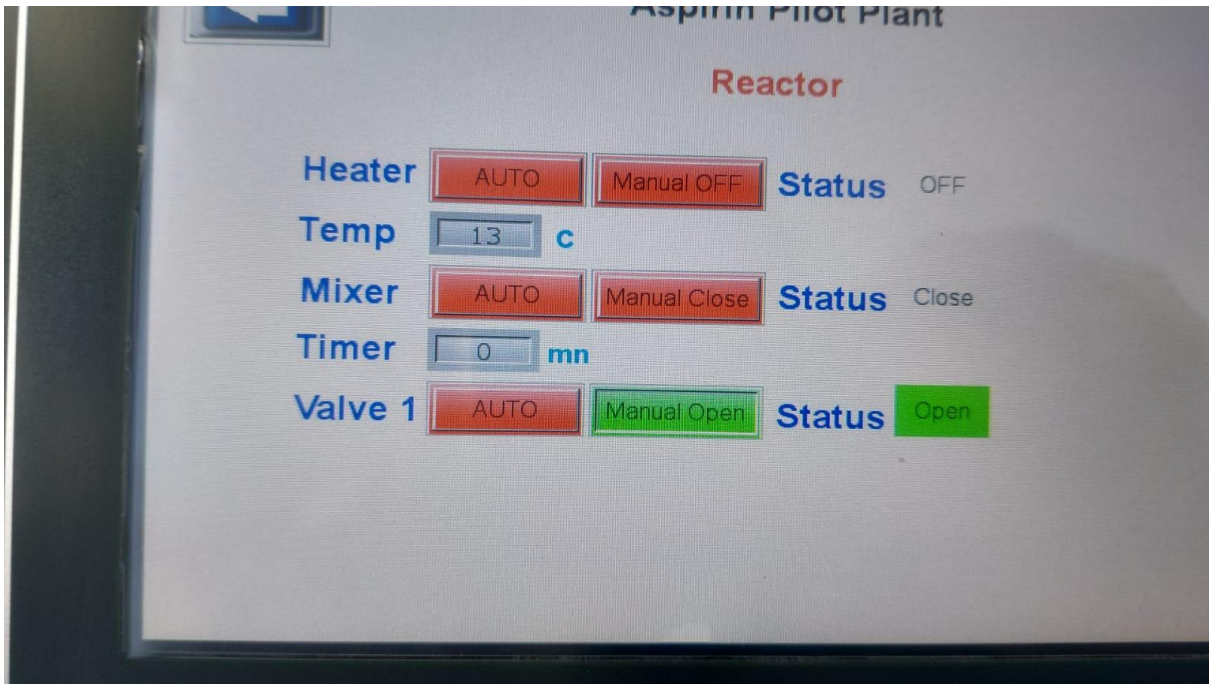
Video 2 water test 28.11.23.mp4



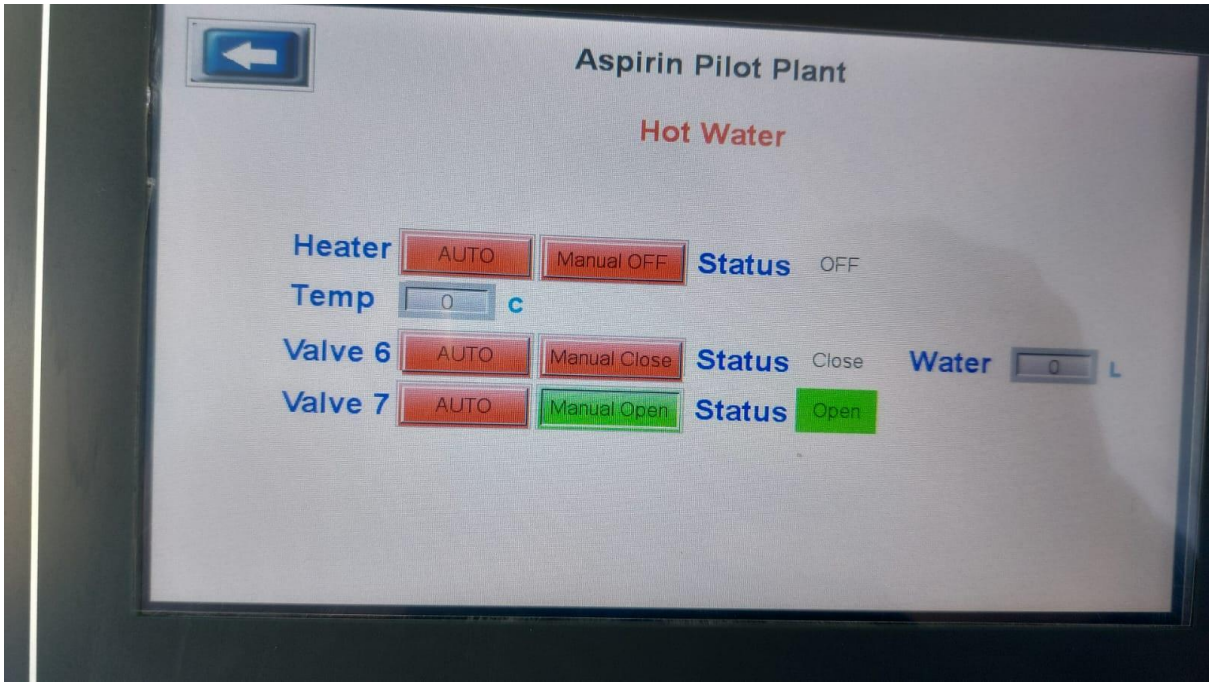
1. Overview of control panel system for Aspirin pilot plant



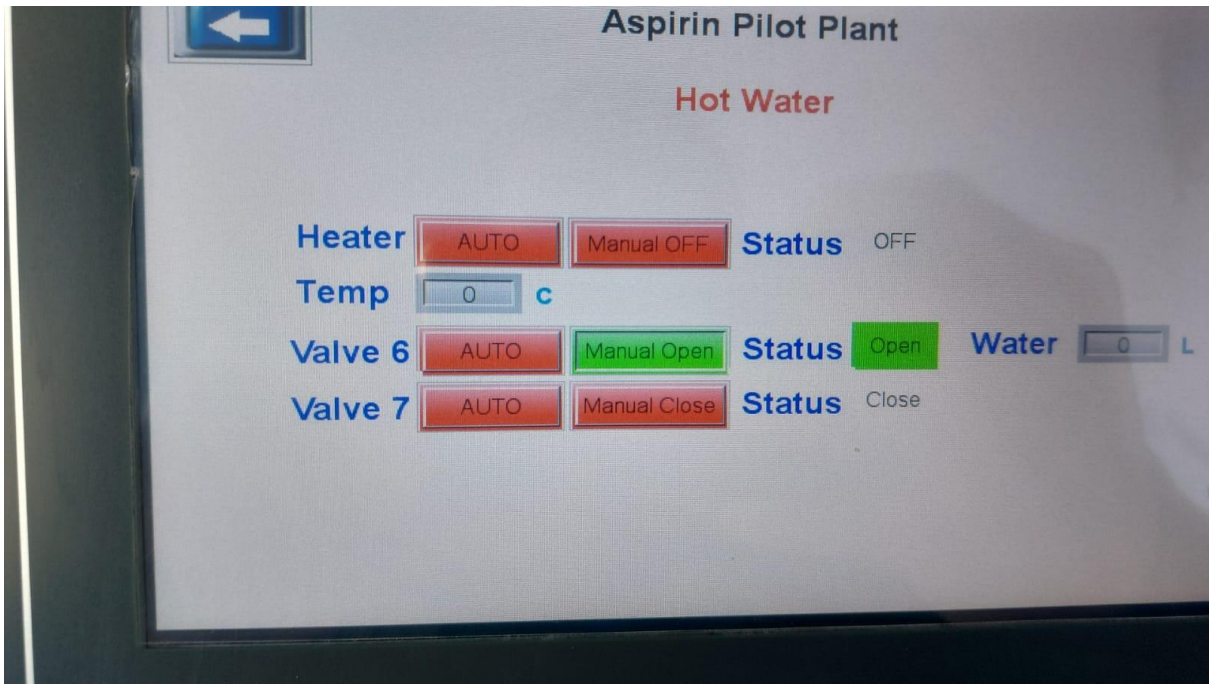
2. Turn ON the mixer by switching manually to mix the solution (PS: in this test we used water only)



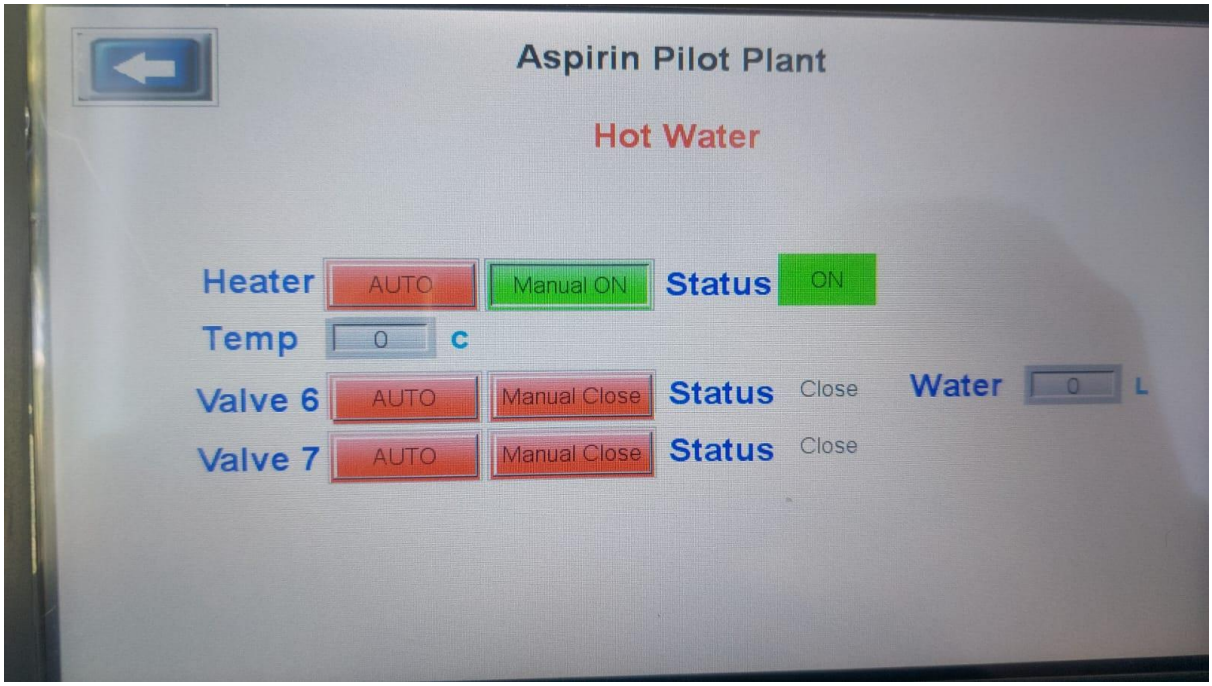
3. Turn ON manually valve 1 to transfer the solution to the Cooler (tank 3)



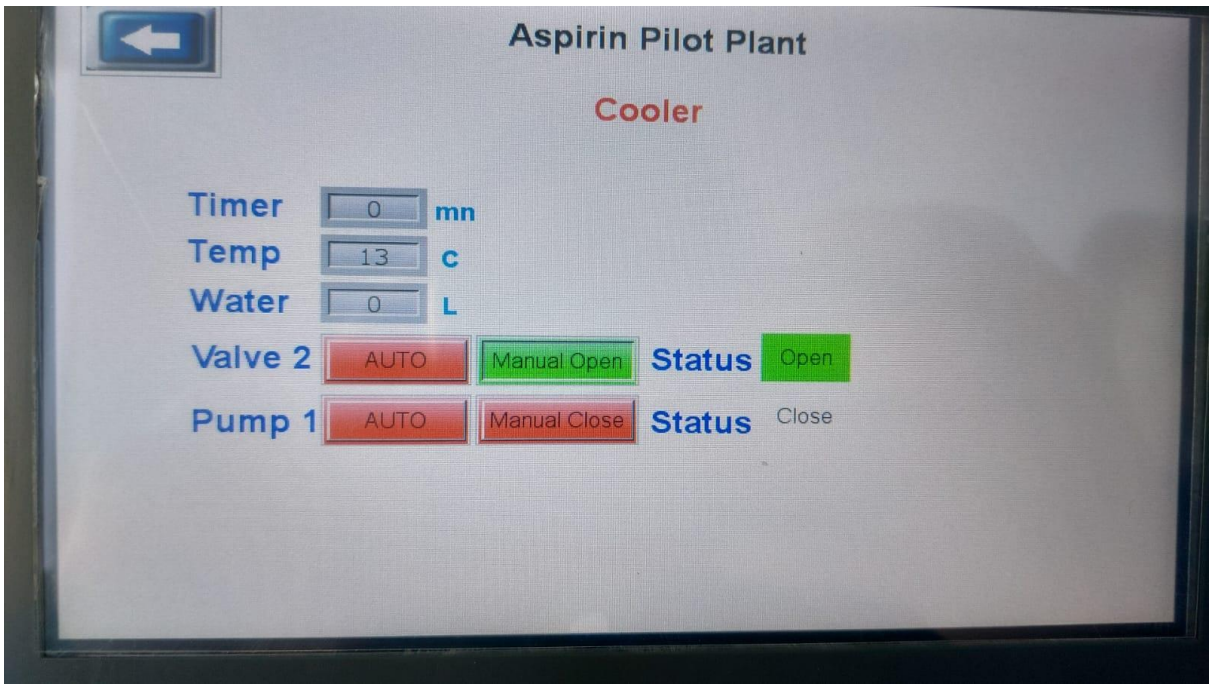
4. Turn ON manually valve 7 to transfer distilled water from Hot water tank (tank 2) to filter (tank 5)



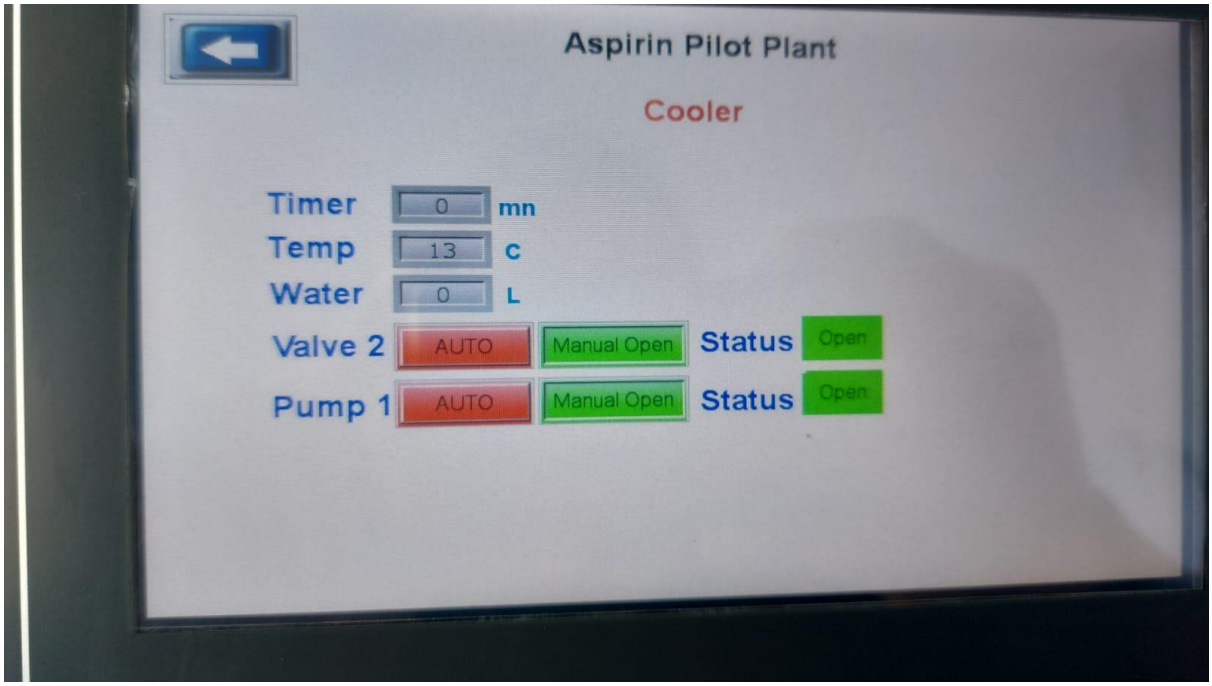
5. Turn ON manually valve 6 to transfer distilled water from Hot water tank (tank 2) to Cooler (tank 3) (PS: there's a flow sensor to determine the quantity needed of distilled water)



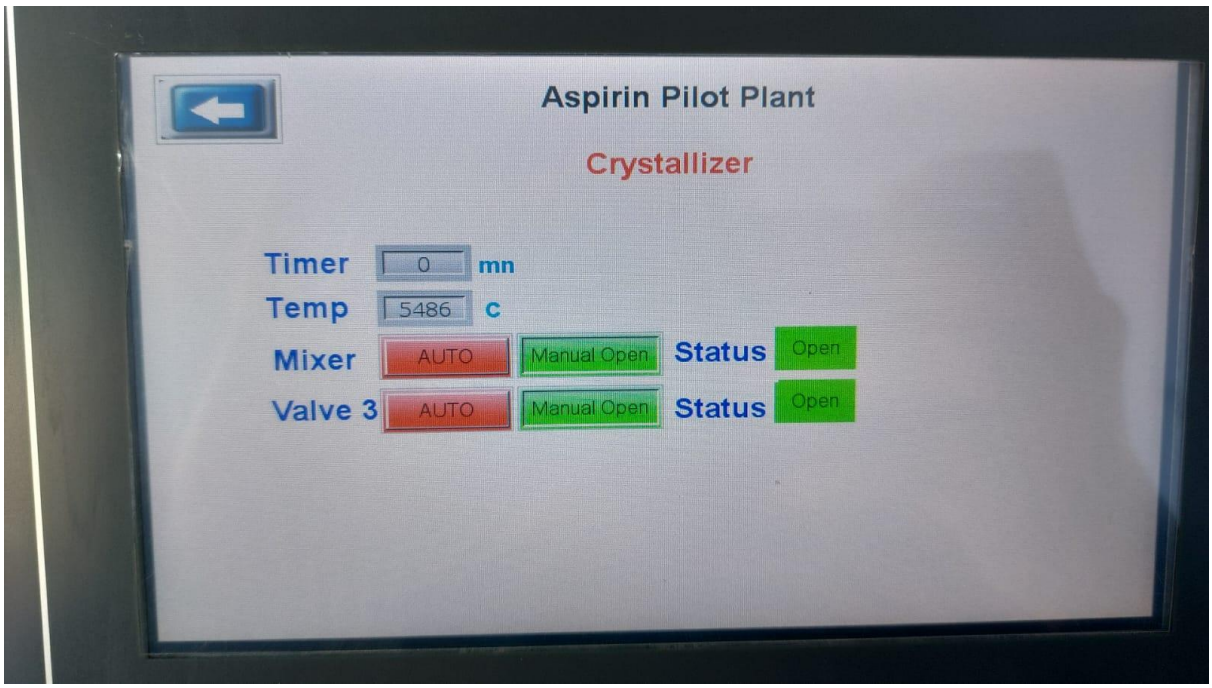
6. Turn ON manually the heater to warm up distilled water needed (PS: there's a temperature sensor to determine the temperature of DW required)



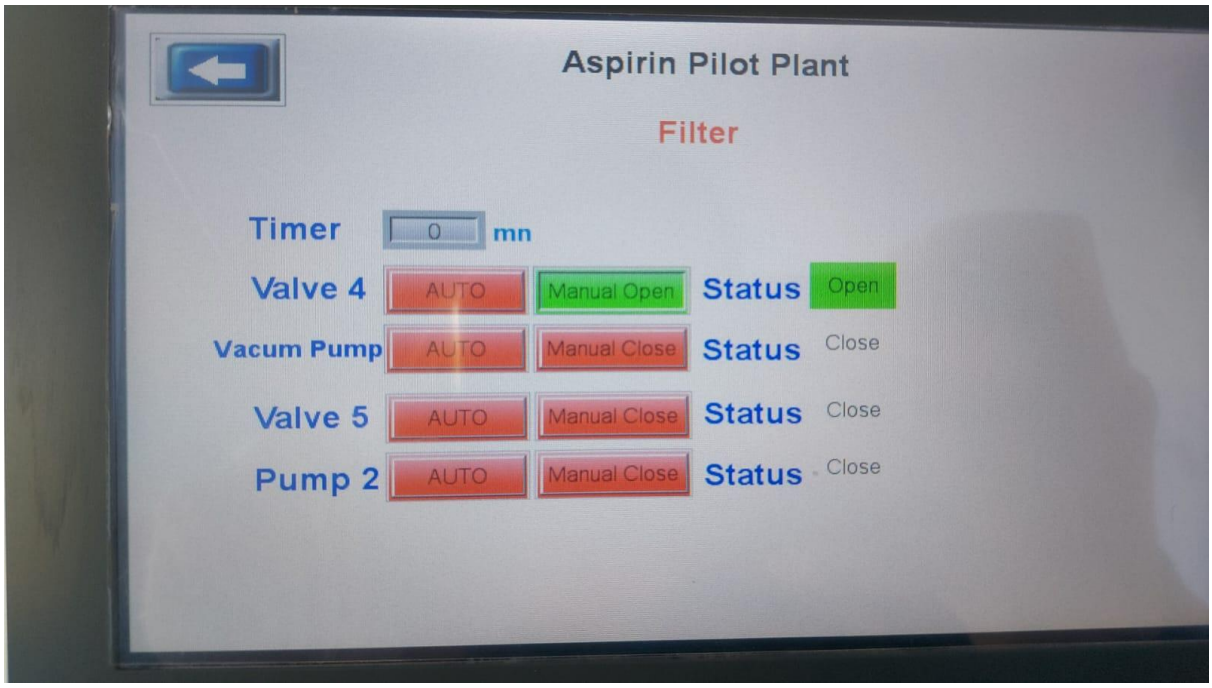
7. Turn ON manually valve 2 to transfer the mixture (Water) from the cooler (tank 3) to crystallizer (tank 4)



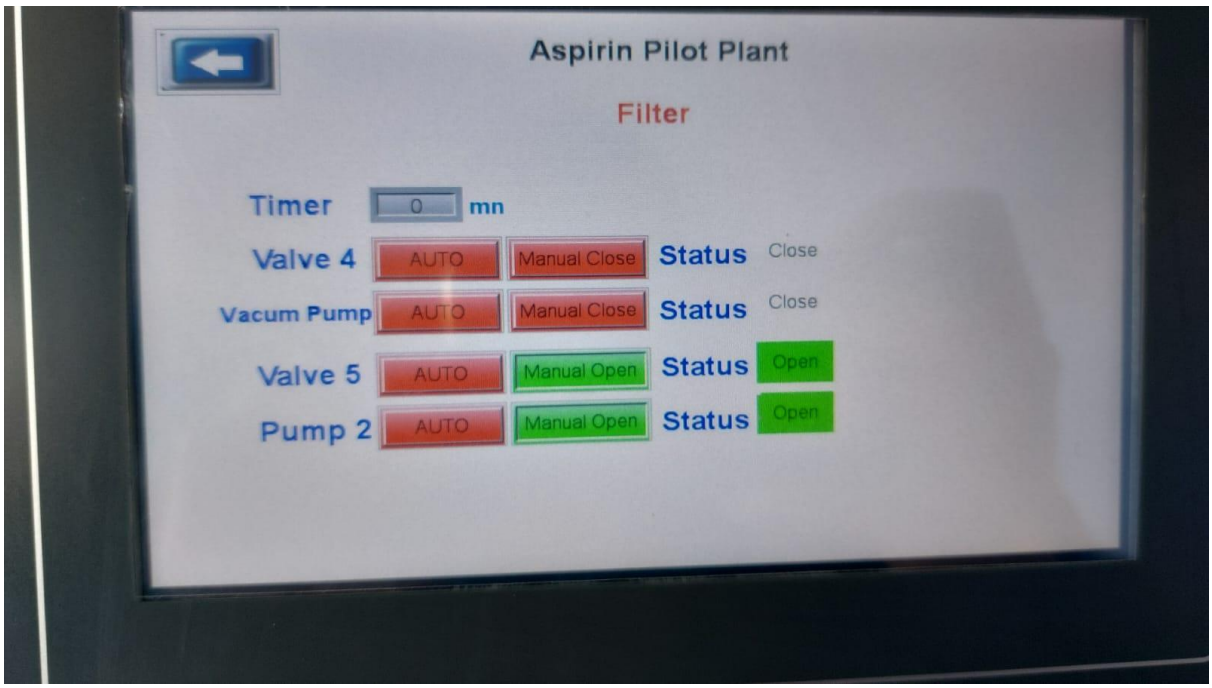
8. Turn ON manually pump 1 to transfer the mixture (Water) from the cooler (tank 3) to crystallizer (tank 4)



9. Turn ON munally 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)

17.5.2 004: 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)

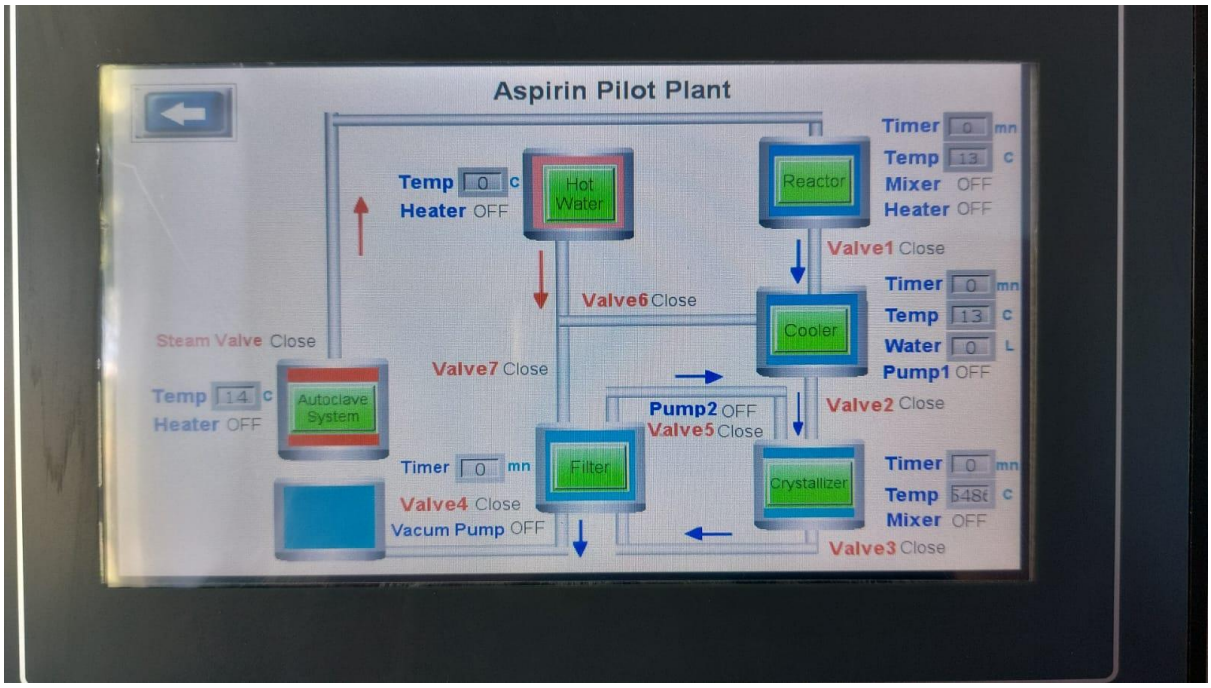
Open the valve V3 (tank 4: Crystallizer)	Open the valve V3 to transfer the Water from crystallizer (tank 4) to the filter (tank 5) (crystallizer---->crystallizer valve V3)	The Water transferred to the filter (tank 5)
Open the valve V7 (TANK 3: HOT WATER)	Open the valve V7 to add water from HOT WATER 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 breaked need to release the pressure from the sensor and we need to make a vent for air pressure in tank 3 (Cooler).

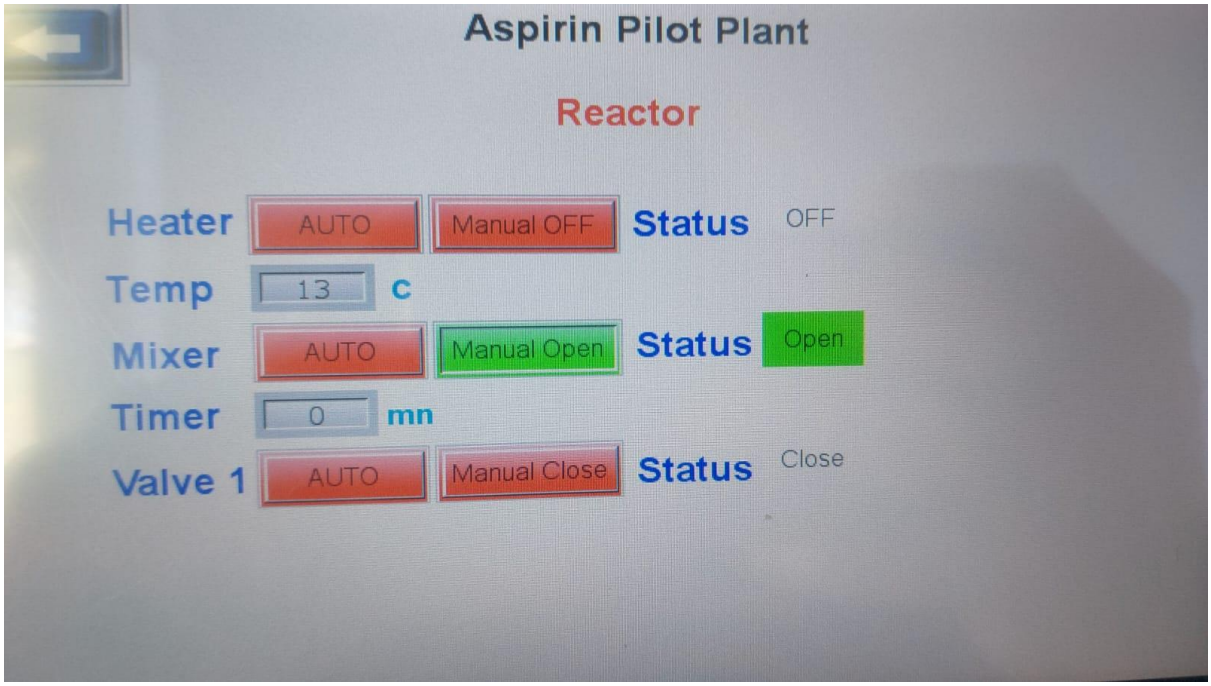
in the next test we will fix all those problems and try another test with water only.

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

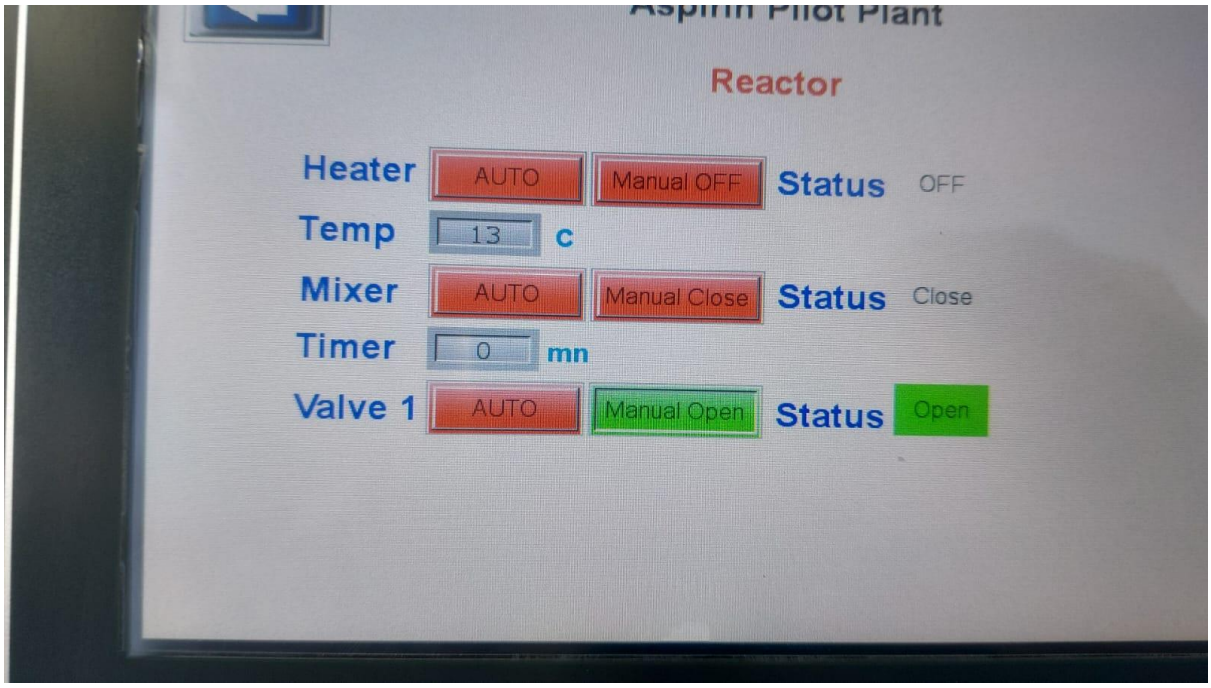
aspirin pilot plant test water only 003 vi



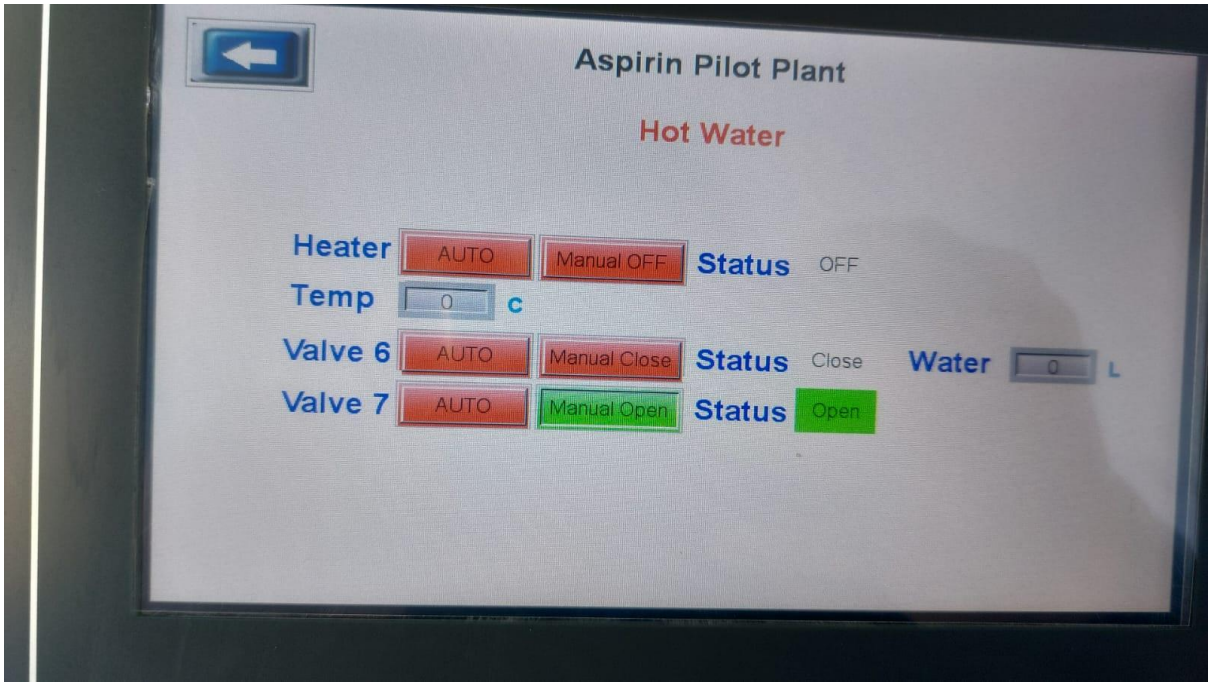
1. Overview of control panel system for Aspirin pilot plant



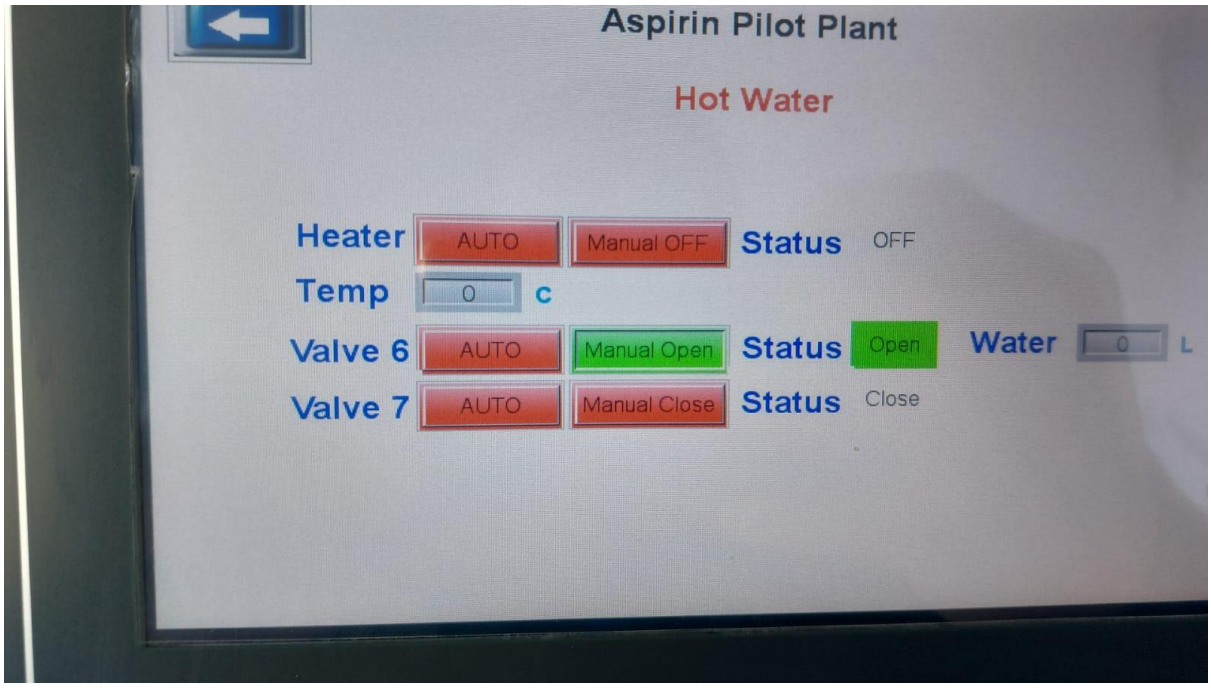
2. Turn ON the mixer by switching manually to mix the solution (PS: in this test we used water only)



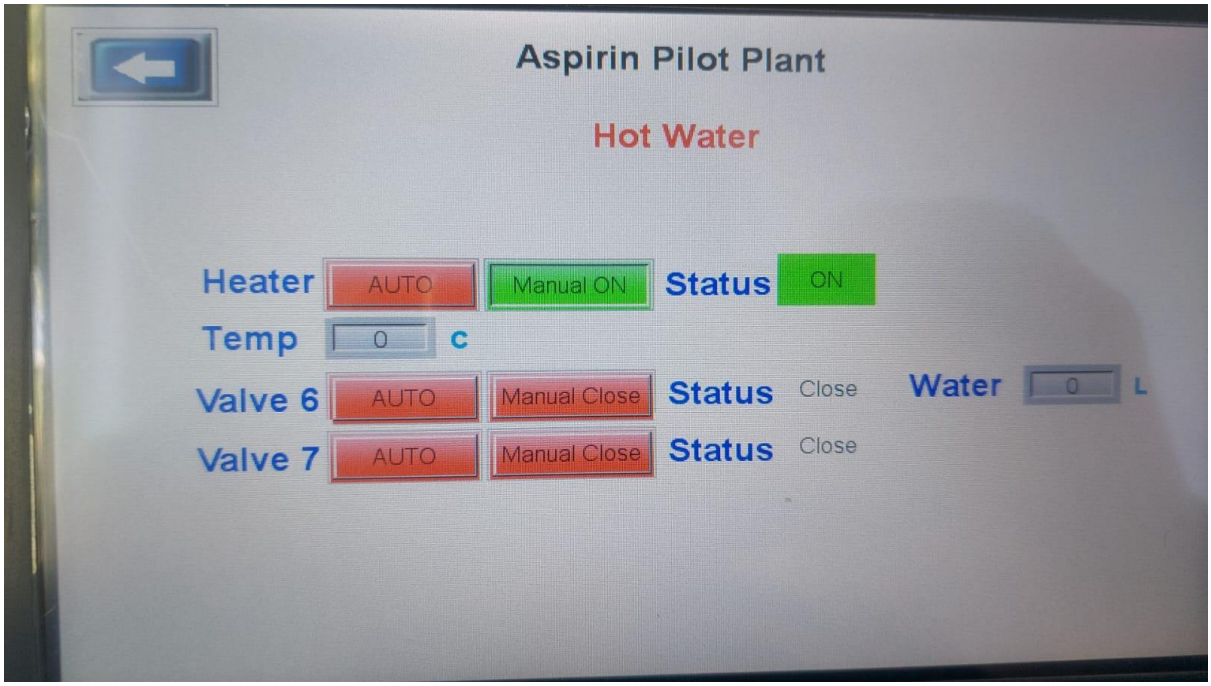
3. Turn ON manually valve 1 to transfer the solution to the Cooler (tank 3)



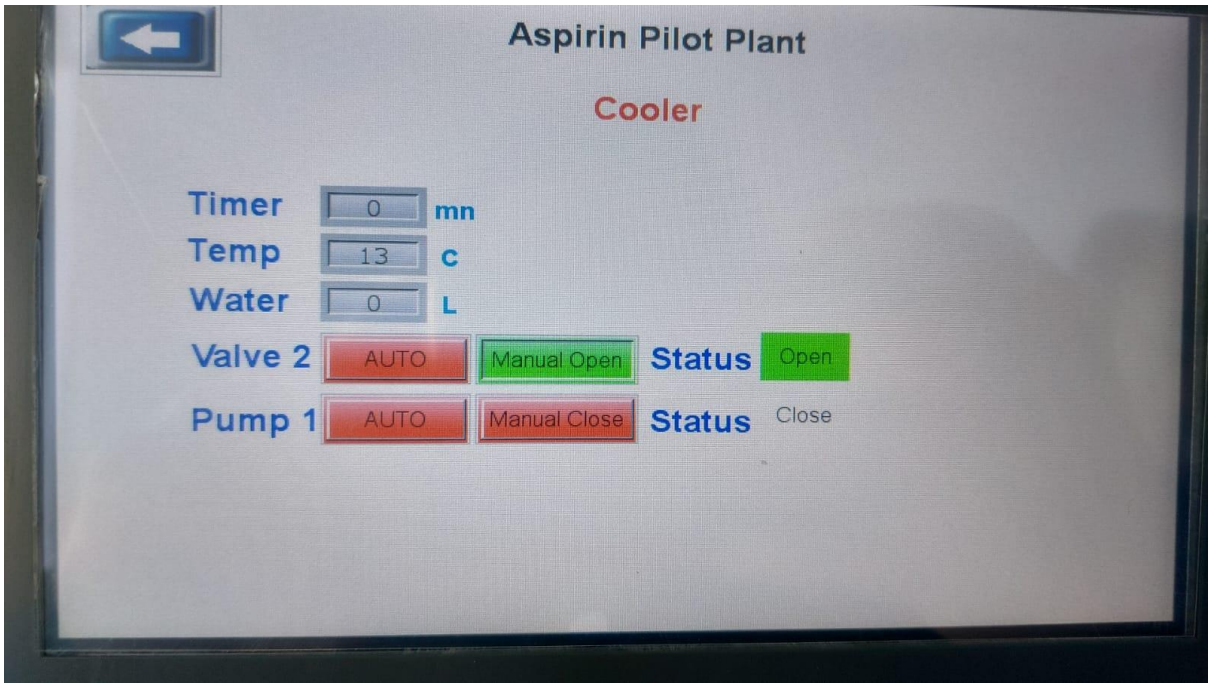
4. Turn ON manually valve 7 to transfer distilled water from Hot water tank (tank 2) to filter (tank 5)



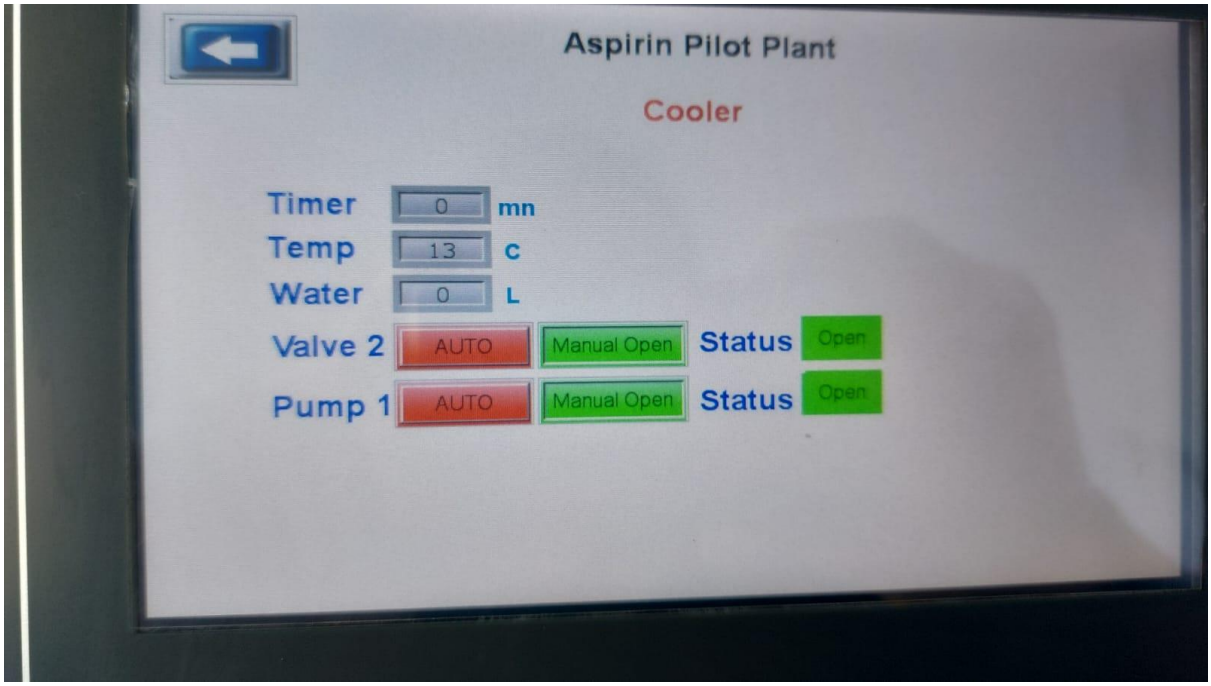
5. Turn ON manually valve 6 to transfer distilled water from Hot water tank (tank 2) to Cooler (tank 3) (PS: there's a flow sensor to determine the quantity needed of distilled water)



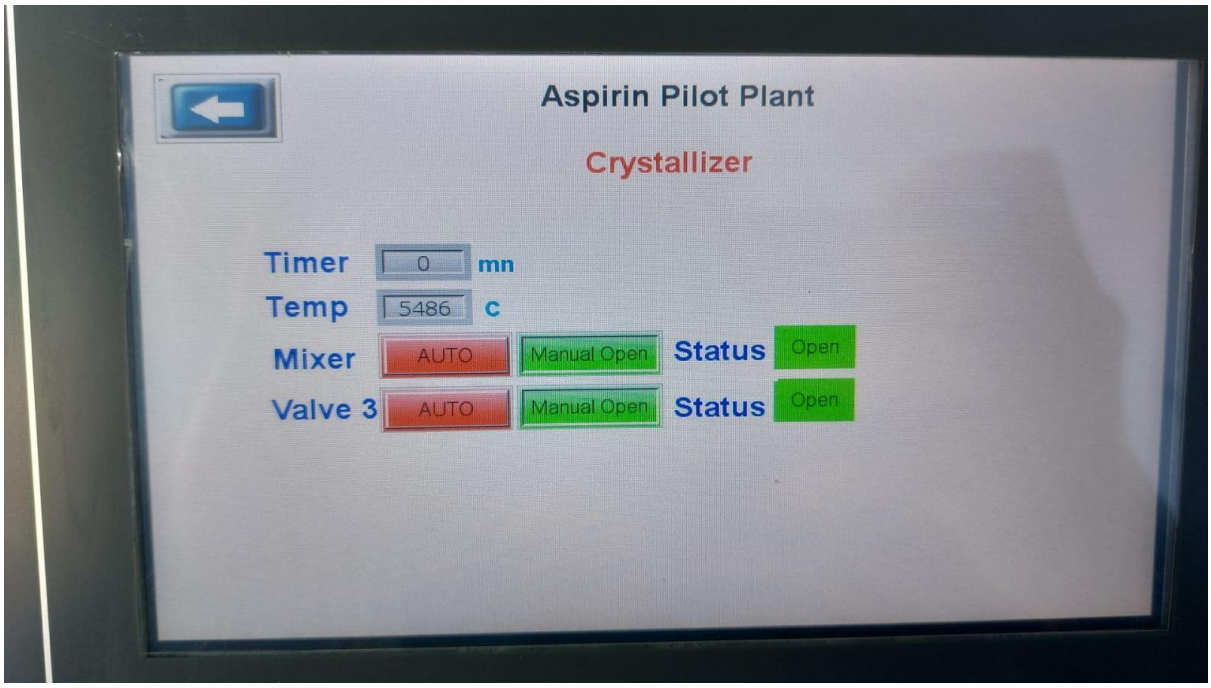
6. Turn ON manually the heater to warm up distilled water needed (PS: there's a temperature sensor to determine the temperature of DW required)



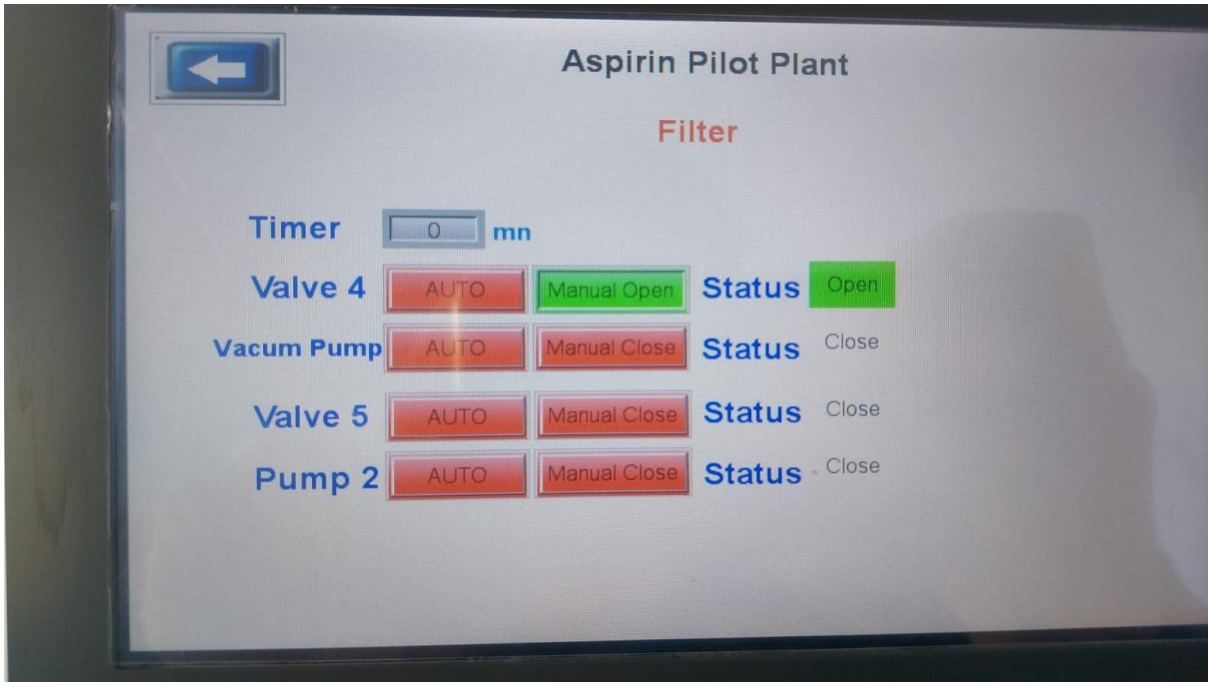
7. Turn ON manually valve 2 to transfer the mixture (Water) from the cooler (tank 3) to crystallizer (tank 4)



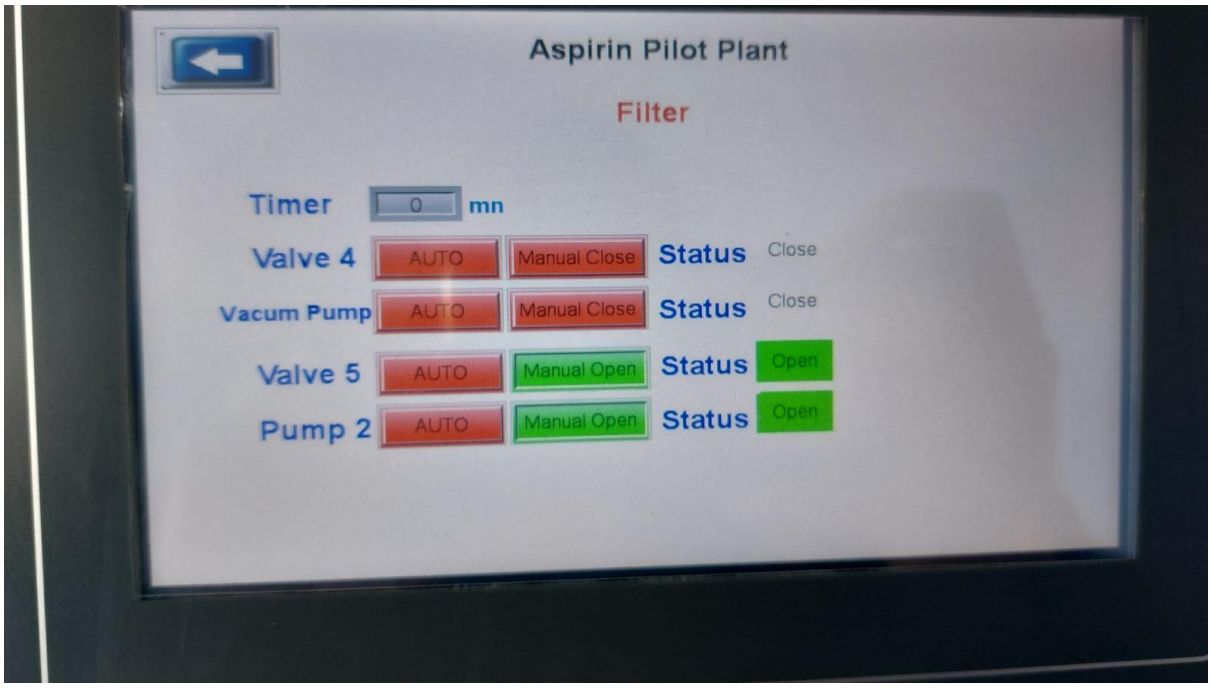
8. Turn ON manually pump 1 to transfer the mixture (Water) from the cooler (tank 3) to crystallizer (tank 4)



9. Turn ON munally 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)



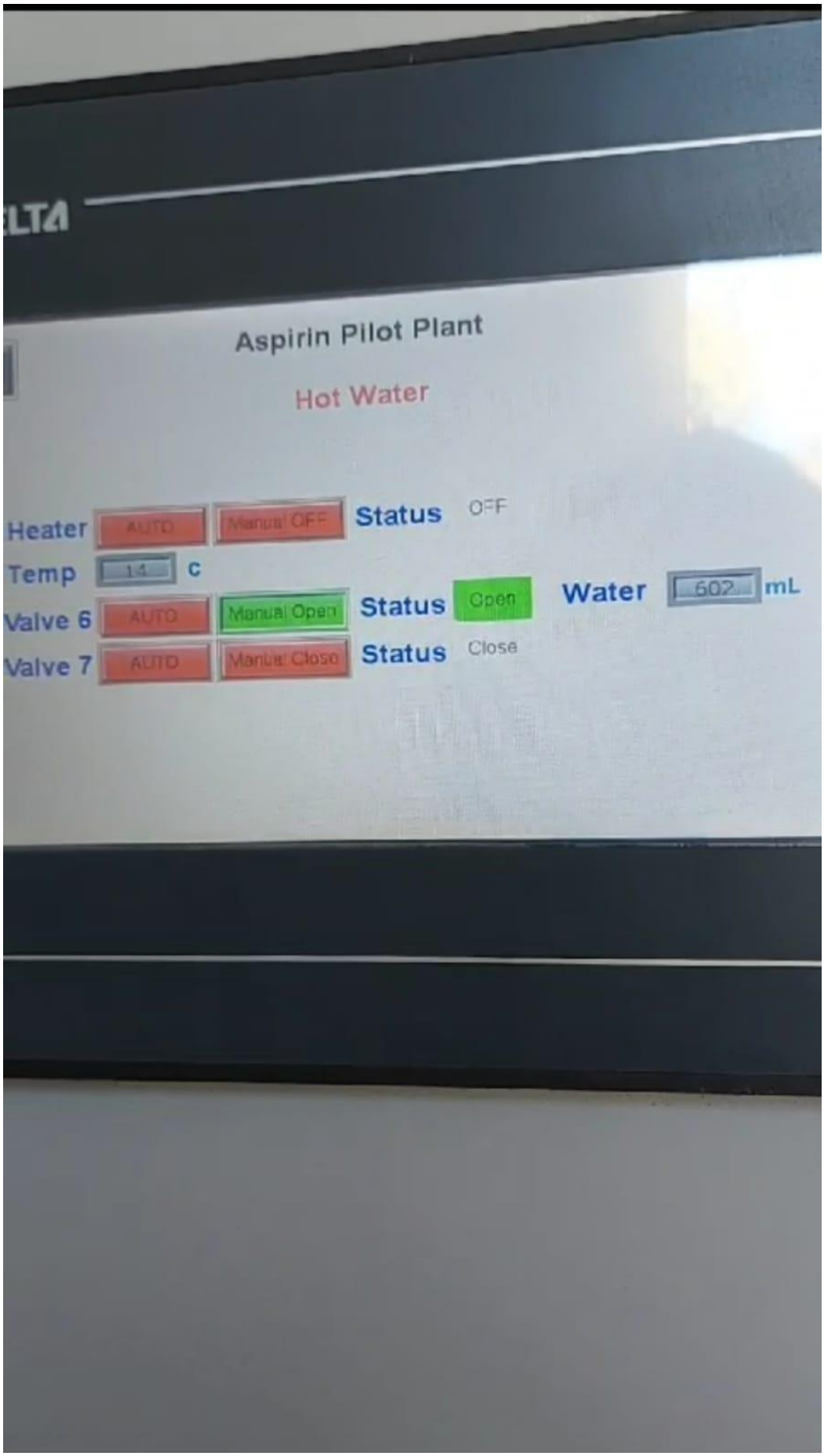
11. Turn ON valve 5 and pump 2 to transfer the mixture (water) from Filter (tank 5) to crystallizer (tank 4) to recrystallization (PURIFICATION STEP)

17.5.3 005: Aspirin Pilot Plant flow sensor Test

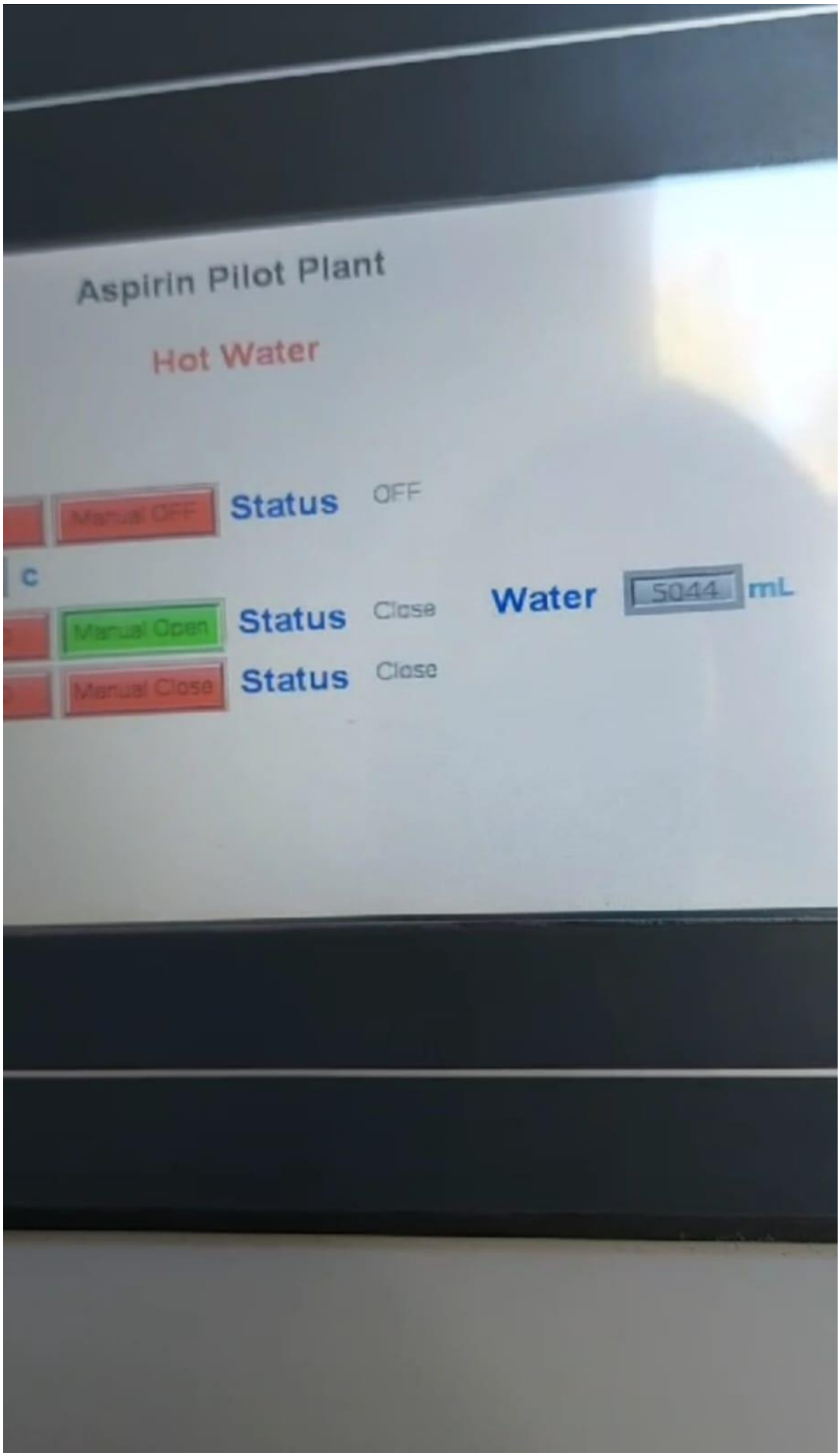
Step	Step Description	Results
Precondition	System is OFF	
TURNING ON the system	Turn ON the control panel	The system is ON
Open the valve 6 (tank 3 : HOT WATER)	Open the valve V6 to add water from **HOT WATER TANK** (tank 3) to the cooler (tank 2) (HOt WATER TANK---->HOT WATER TANK valve V6)	The water 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


Aspirin Pilot Plant
Hot Water
Heater
AUTO
Manual OFF
Status
OFF
Temp
14
C
Valve 6
AUTO
Manual Open
Status
Open
Water
602
mL
Valve 7
AUTO
Manual Close
Status
Close

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
Manual OFF
Status
OFF
C
Manual Open
Status
Close
Water
5044
mL
Manual Close
Status
Close

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

17.5.4 006: Aspirin Production System Test (Water test)

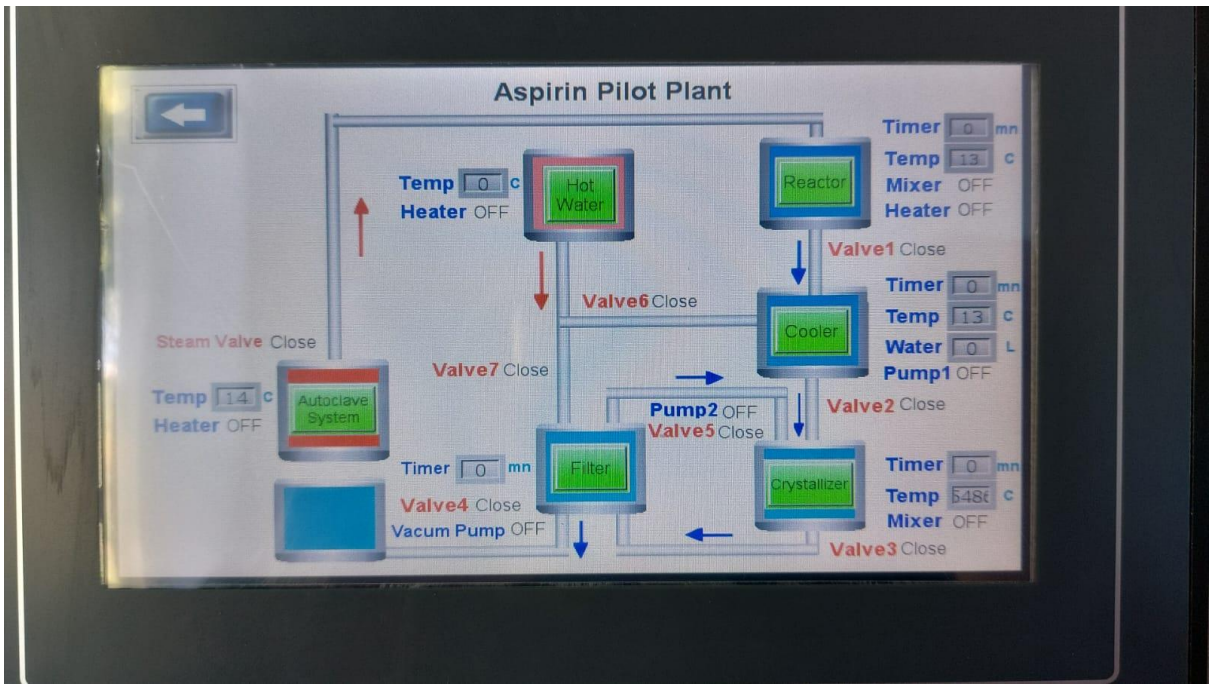
Step	Step Description	Results
Precondition	System is OFF	
TURNING ON the system	Turn ON the control panel	The system is ON
Switch on the mixer 1 (tank 1 : Reactor)	Turn on the mixer 1 from the control panel (Reactor--->Mixer ON)	Mixer 1 is ON and start Mixing the Water (Reactor)
Open the valve V1 (tank1 : 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)	The Water transferred to the filter (tank 5)

	(crystallizer---->crystallizer valve V3)	
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	

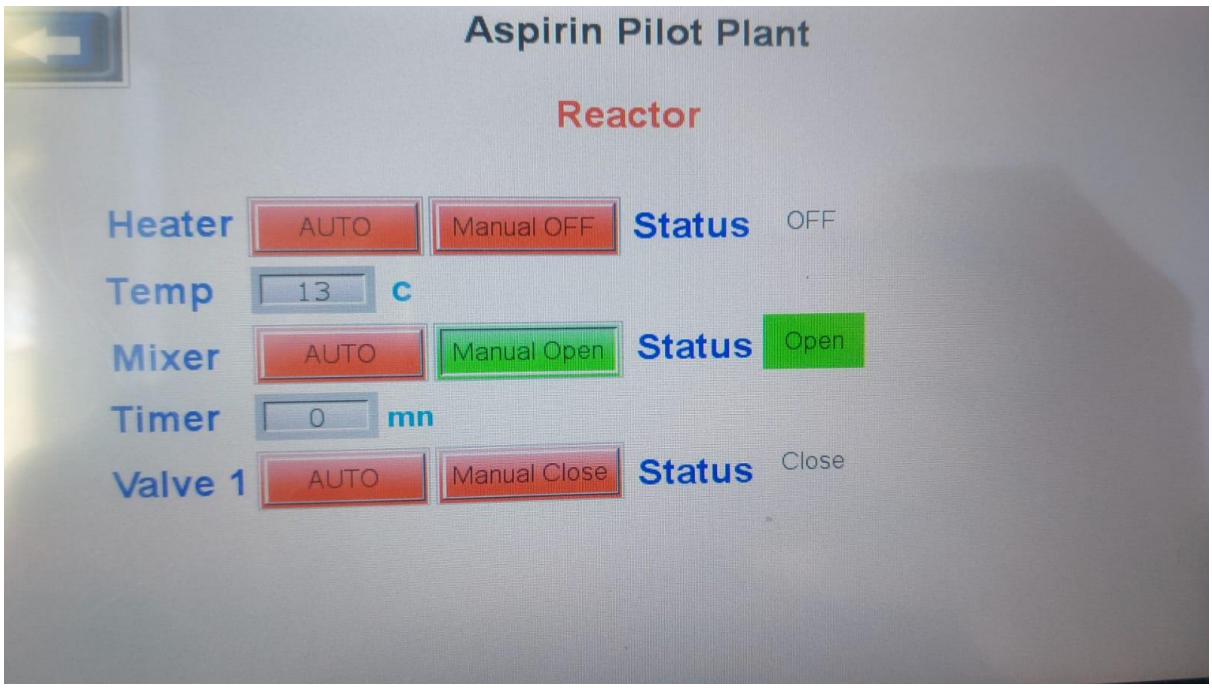
video006: video 3.1 testing all mixers pumps and control system (water only) 14/12/2023

test video 3.1 system test 14.12.23.mp4

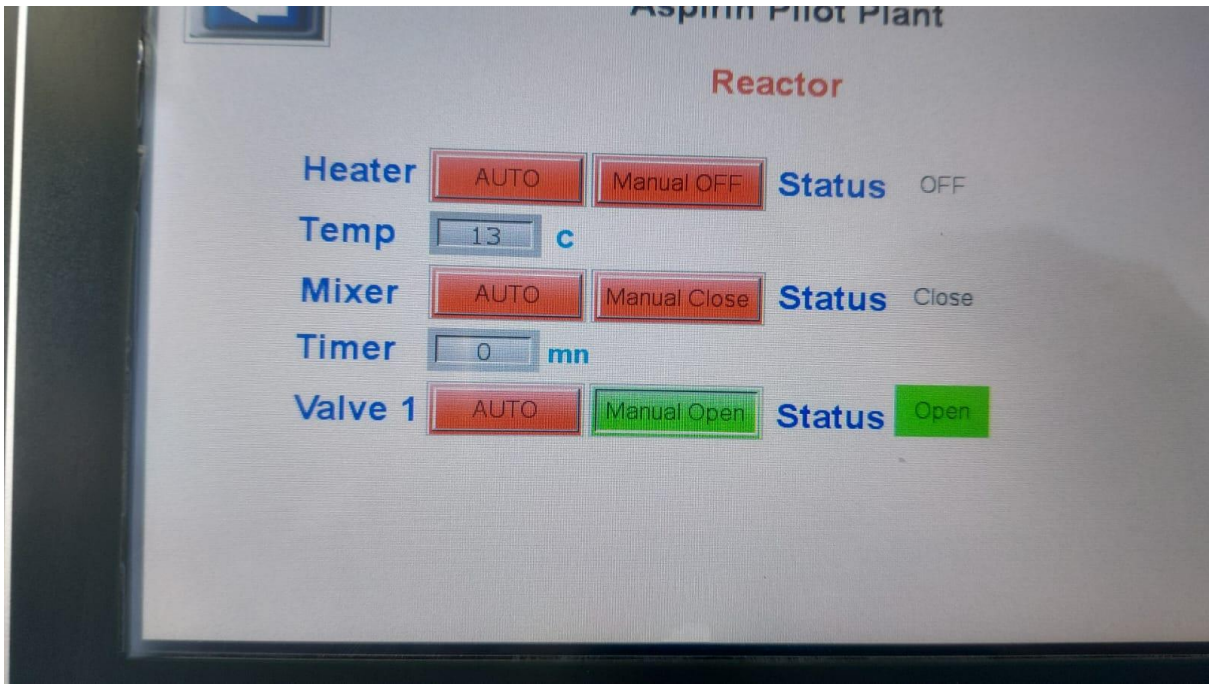
Overview: Mixers, Valves and pumps are working well and this test was enough to prove this, so for the next step we should try the same test with the chemicals reagents



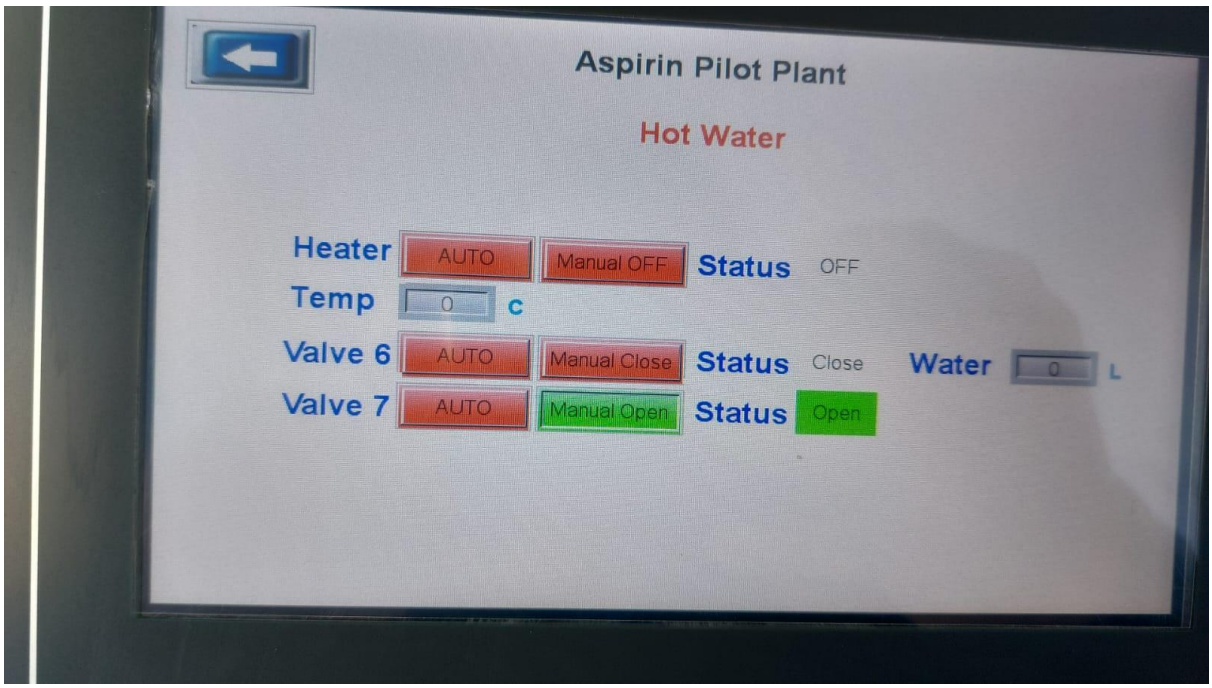
1. Overview of control panel system for Aspirin pilot plant



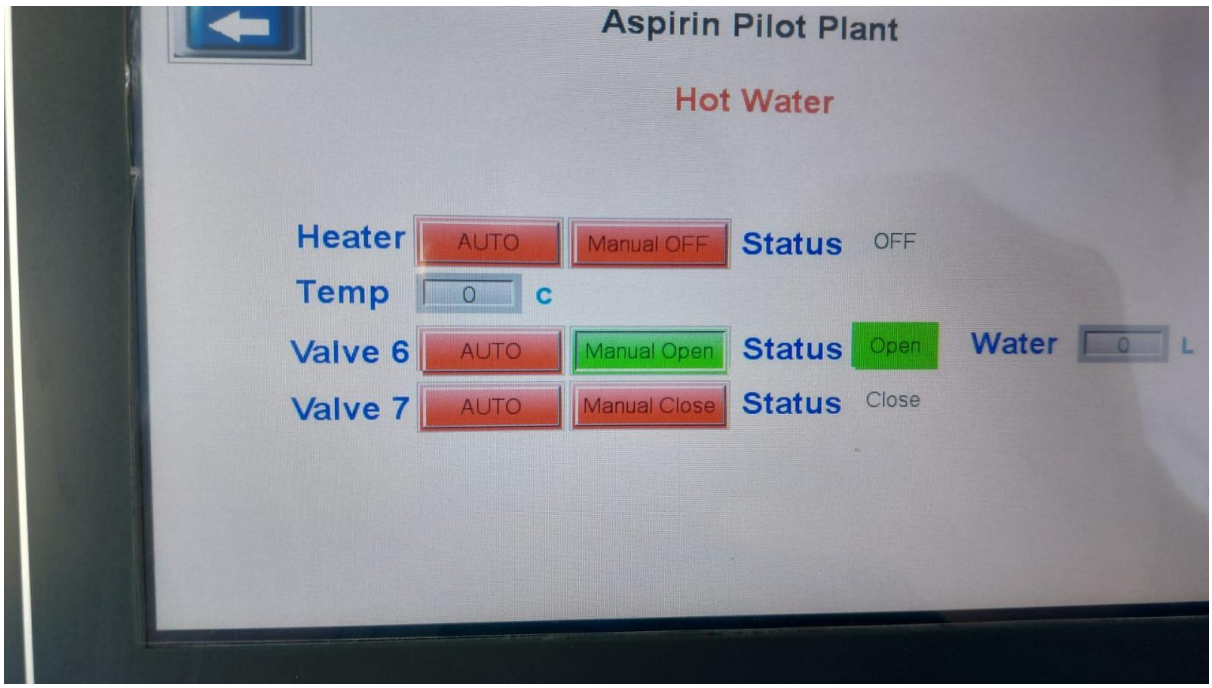
2. Turn ON the mixer by switching manually to mix the solution (PS: in this test we used water only)



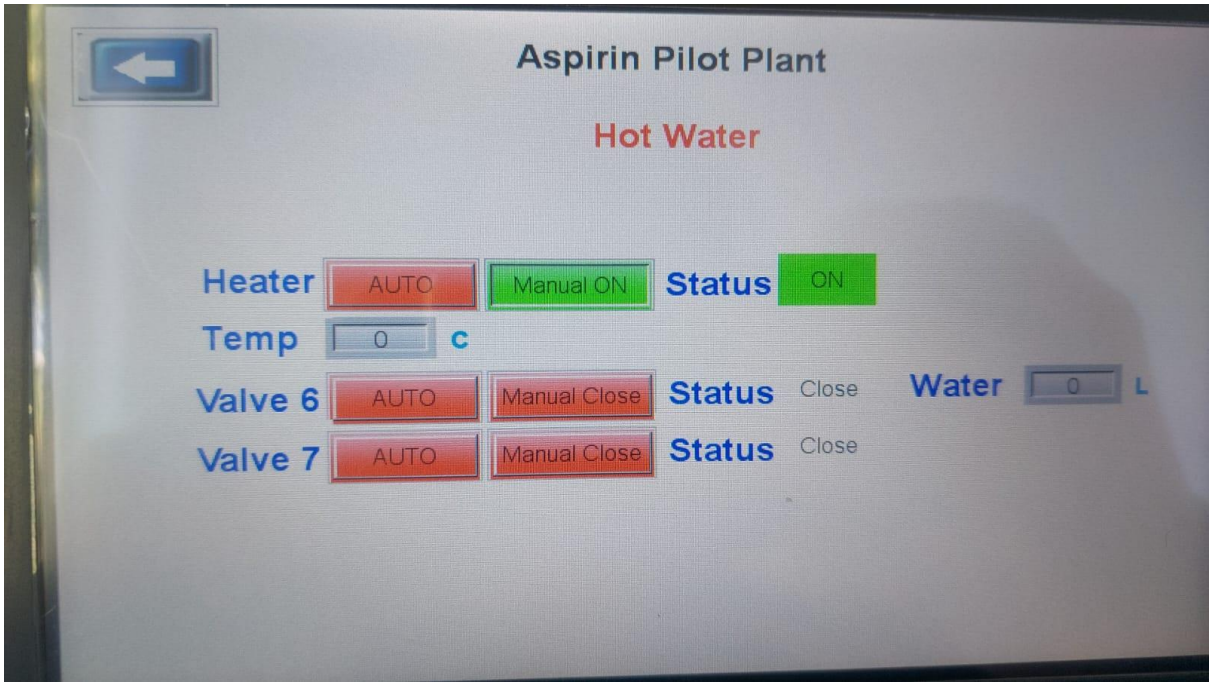
3. Turn ON manually valve 1 to transfer the solution to the Cooler (tank 3)



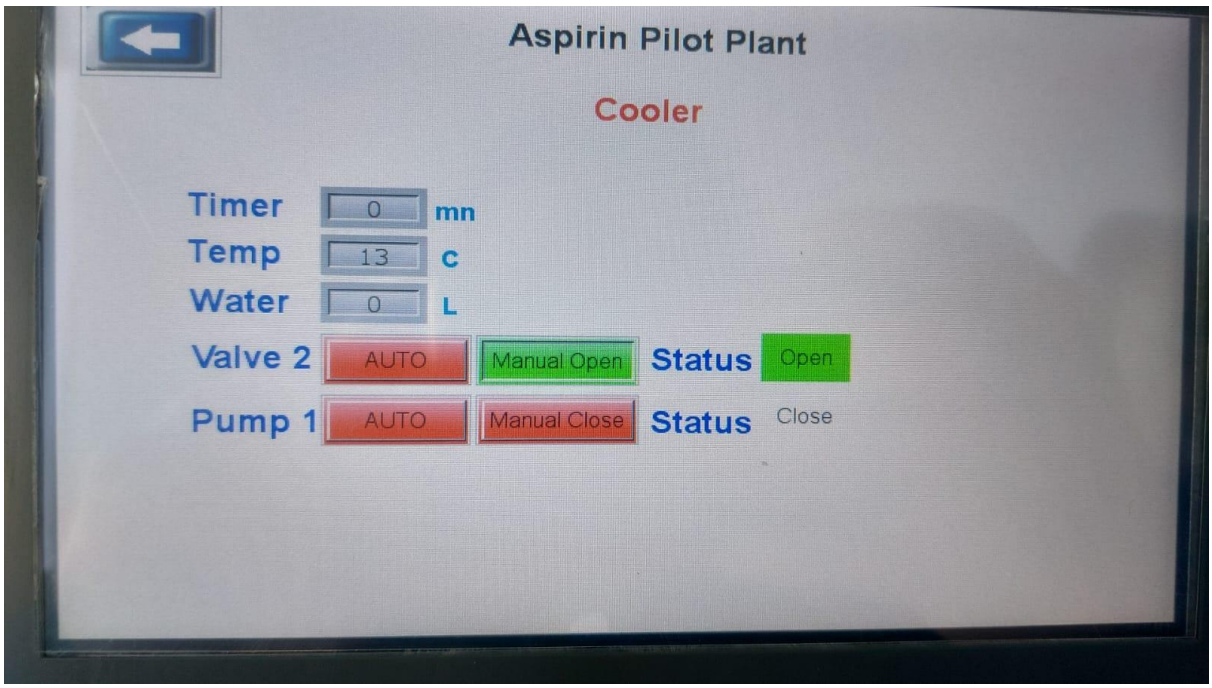
4. Turn ON manually valve 7 to transfer distilled water from Hot water tank (tank 2) to filter (tank 5)



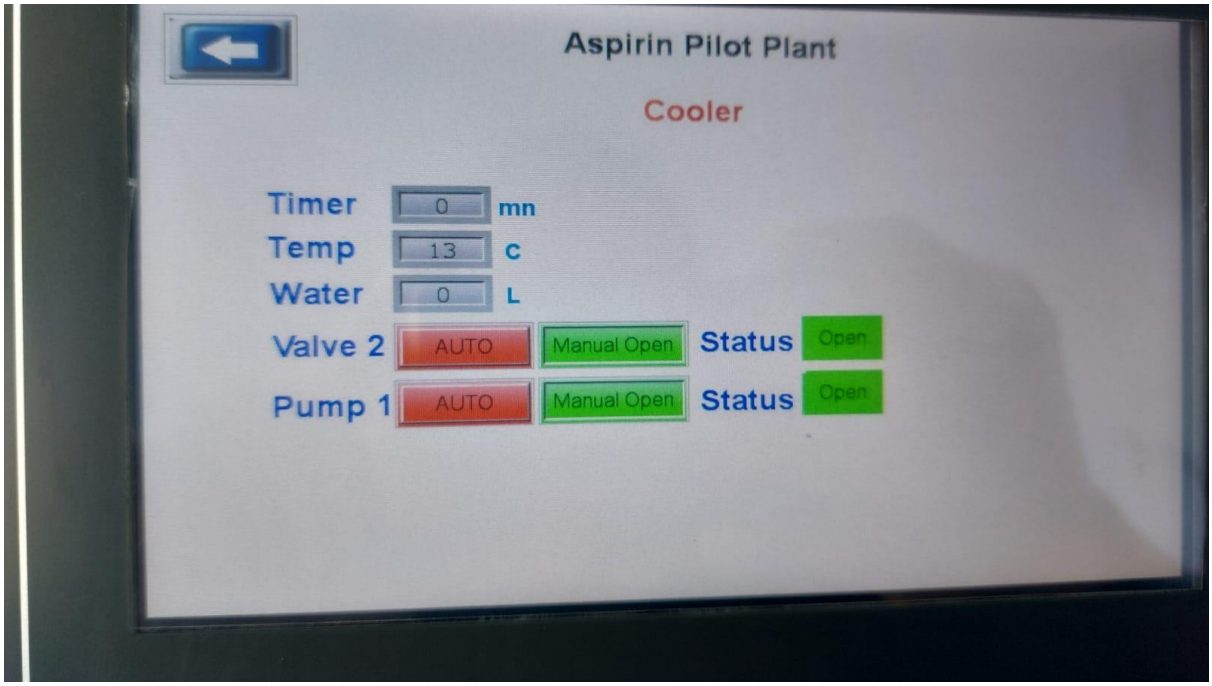
5. Turn ON manually valve 6 to transfer distilled water from Hot water tank (tank 2) to Cooler (tank 3) (PS: there's a flow sensor to determine the quantity needed of distilled water)



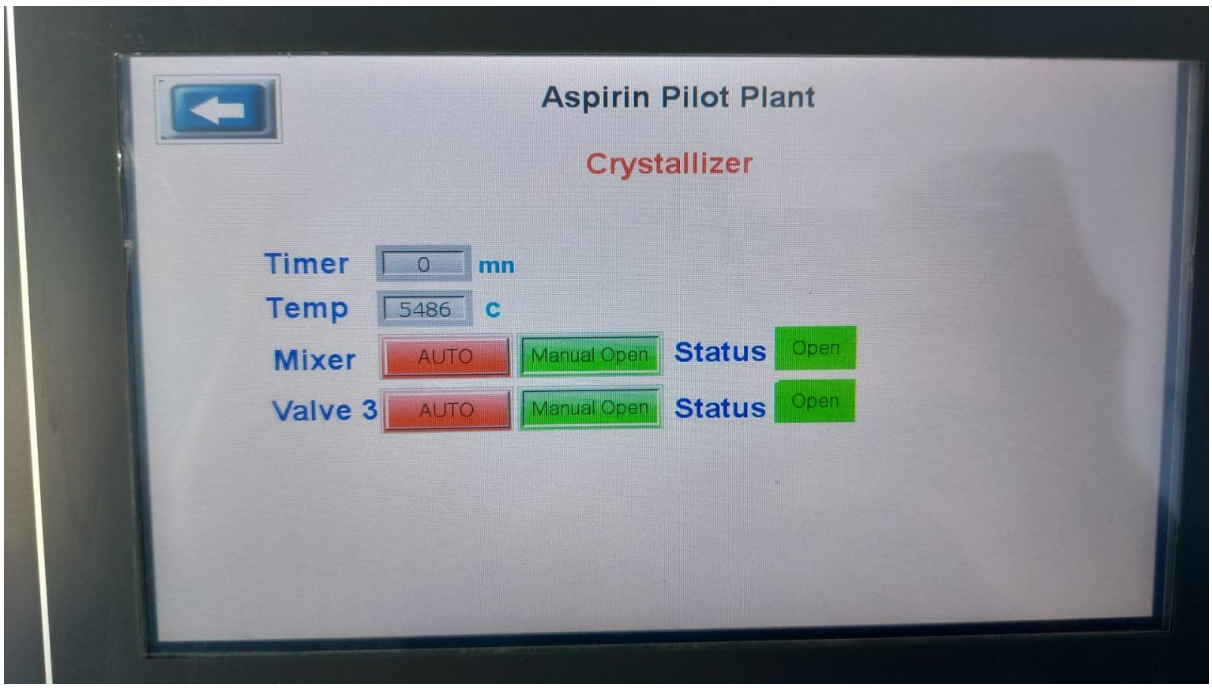
6. Turn ON manually the heater to warm up distilled water needed (PS: there's a temperature sensor to determine the temperature of DW required)



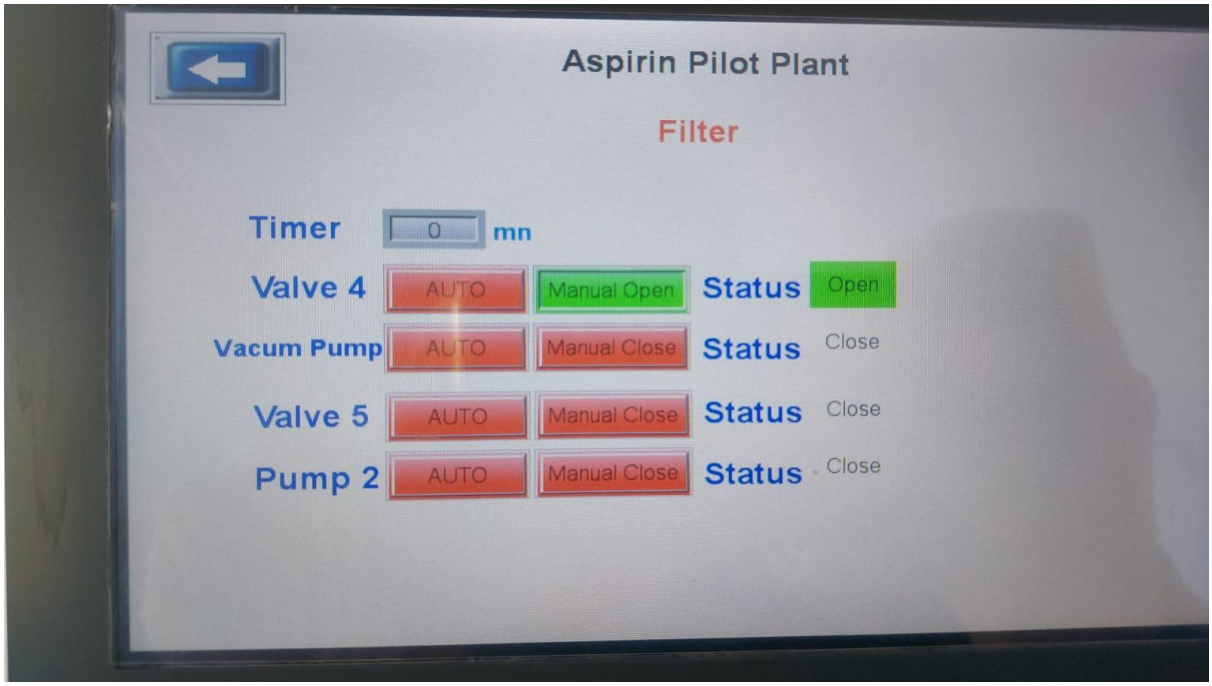
7. Turn ON manually valve 2 to transfer the mixture (Water) from the cooler (tank 3) to crystallizer (tank 4)



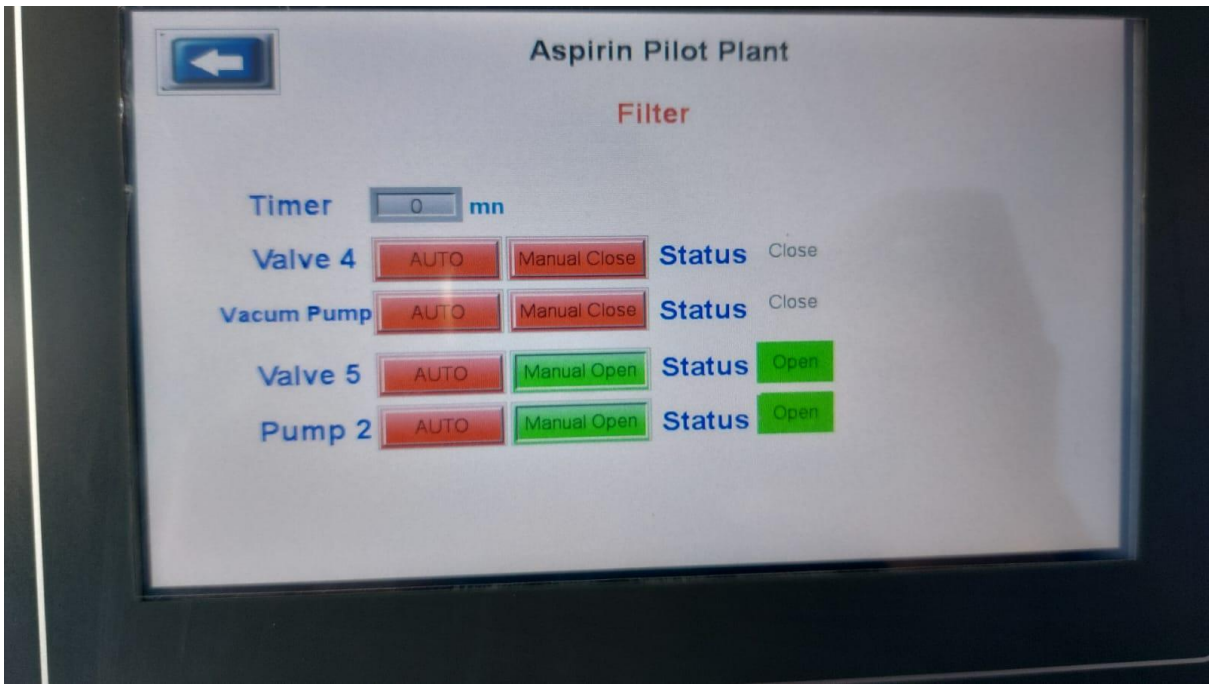
8. Turn ON manually pump 1 to transfer the mixture (Water) from the cooler (tank 3) to crystallizer (tank 4)



9. Turn ON munally 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)



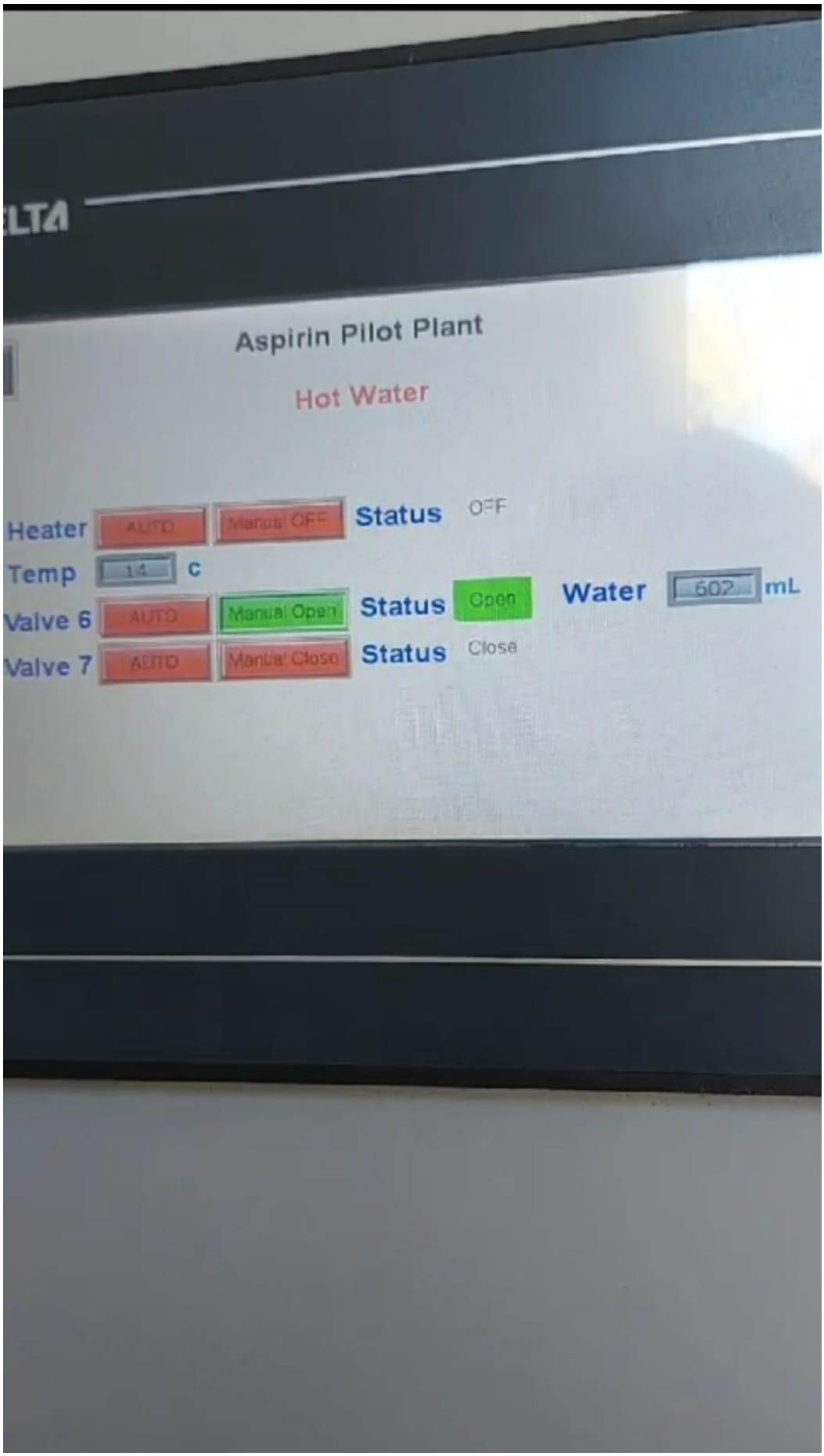
11. Turn ON valve 5 and pump 2 to transfer the mixture (water) from Filter (tank 5) to crystallizer (tank 4) to recrystallization (PURIFICATION STEP)

17.5.5 007: Aspirin Pilot Plant flow sensor Test

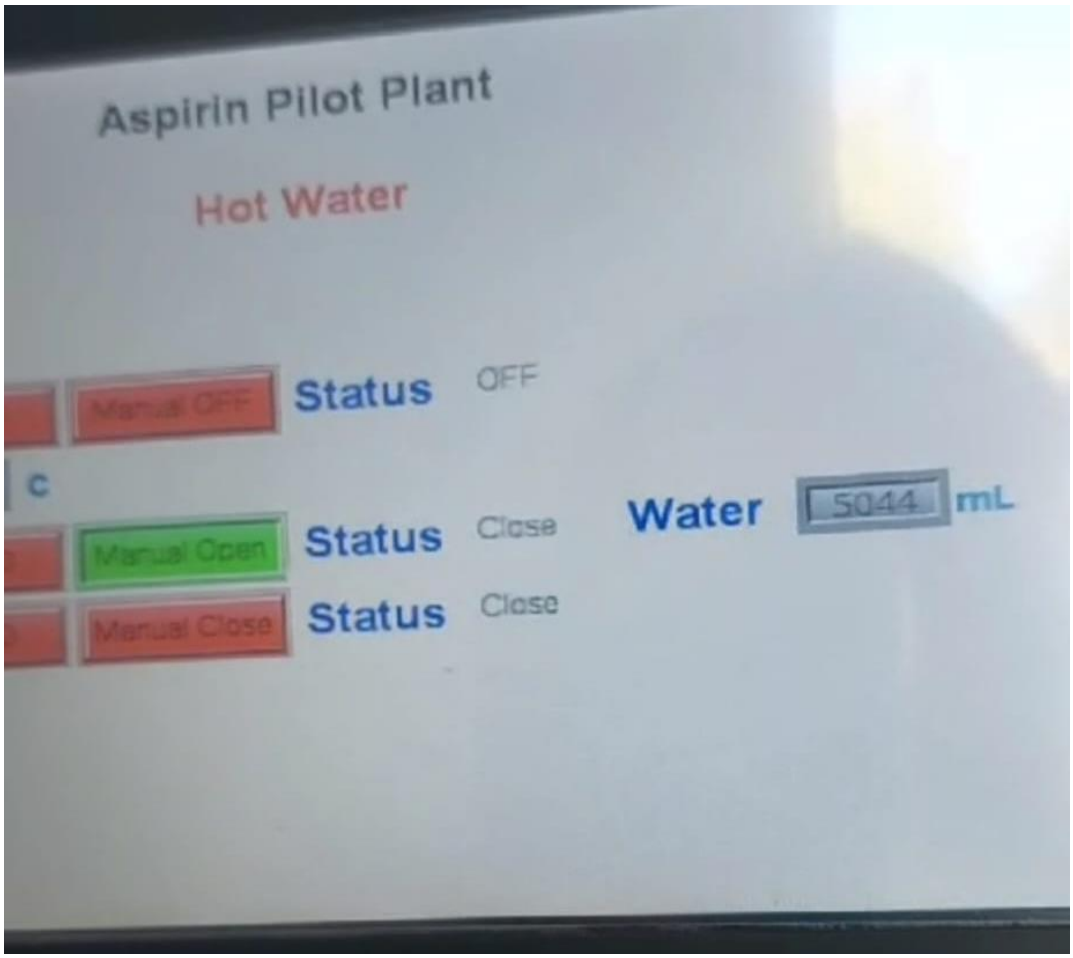
Step	Step Description	Results
Precondition	System is OFF	
TURNING ON the system	Turn ON the control panel	The system is ON
Open the valve 6 (tank 3 : HOT WATER)	Open the valve V6 to add water from **HOT WATER TANK** (tank 3) to the cooler (tank 2) (HOt WATER TANK---->HOT WATER TANK valve V6)	-The water 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


Aspirin Pilot Plant
Hot Water
Heater
AUTO
Manual OFF
Status
OFF
Temp
14
C
Valve 6
AUTO
Manual Open
Status
Open
Water
602
mL
Valve 7
AUTO
Manual Close
Status
Close

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



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

17.5.6 008: ASPIRIN PILOT PLANT TEST (AUTOCLAVE TEST)

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
Sterilizaiton the tank 5 (Filter)	Open the valve V3 to sterilize tank 4 (Crystallizer) to the tank 5 (Filter) (crystallizer---->crystallizer valve V3)	-No results
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) autocalve 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

Inhalt

17.6 Cleaning/Tableting/Recycling Pilot plant

17.6.1 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
- 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 diffi cult 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.

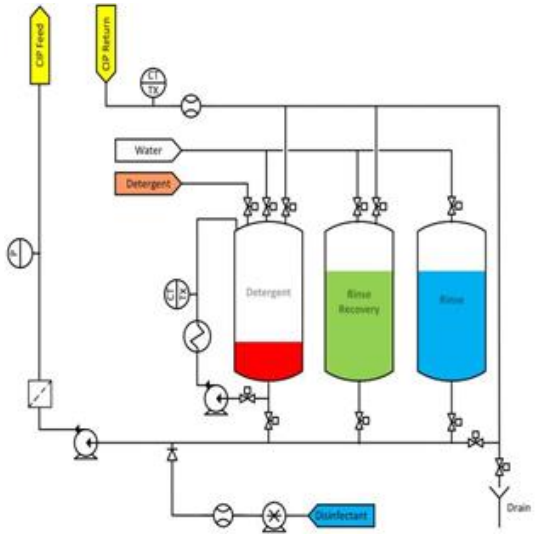
Note! the final rinse water may be recovered and reused as the pre-rinse solution for the next cleaning cycle.

- ***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.
- 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.

17.6.2 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)	R**ole**
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	Giladant

Meth acrylic Acid-Ethyl Acrylate Copolymer (1:1)	10.50 (As dry substance)	Tablet binder, tablet coating agent
Triethyl Citrate	1.05	It allows a slower release of the contents of th 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:

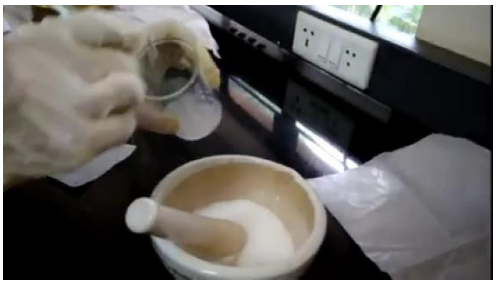
- Chemicals: Aspirin, starch, polyvinyl pyrrolidone, ethanol, magnesium stearate, talc.
- Glass wares: measuring cylinder, beaker, mortar and pestle, granulating sieve…
- Equipment: tray dryer, tablet press.

Procedure:

1. Weight and pass aspirin and starch powder through 60#sieve (size with a 0.0098" (250μm) 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

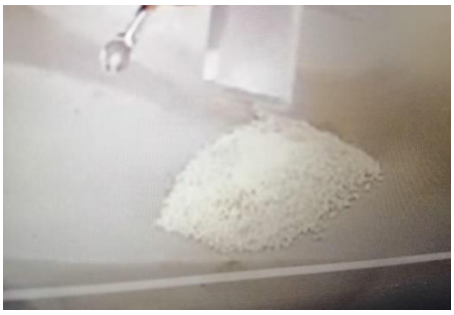


4. Add PVP solution dropwise in mortar to get cohesive mass

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

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



8. 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

17.6.3 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:

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

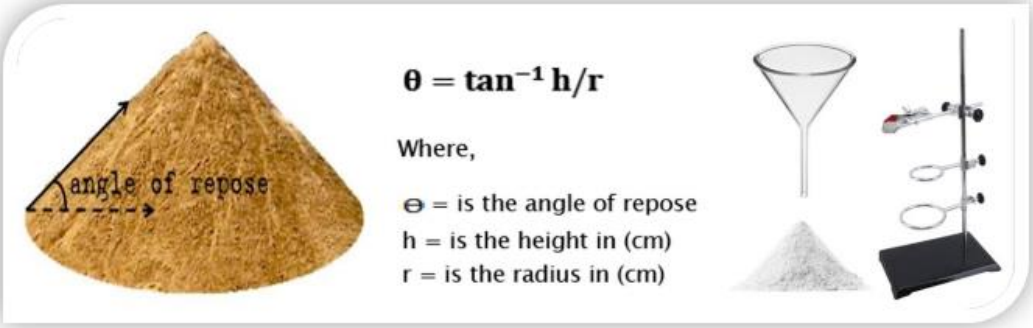
A Hausner Index greater than 1.2 is considered an indication of poor flow but good compressibility and good cohesion.

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 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.

17.6.4 Recycling

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:

$C_7H_6O_3 + H_3O^+ \rightarrow C_7H_7O_3^+ + H_2O$.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:

$CH_3COOH + NaOH \rightarrow CH_3COONa + H_2O$

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 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.

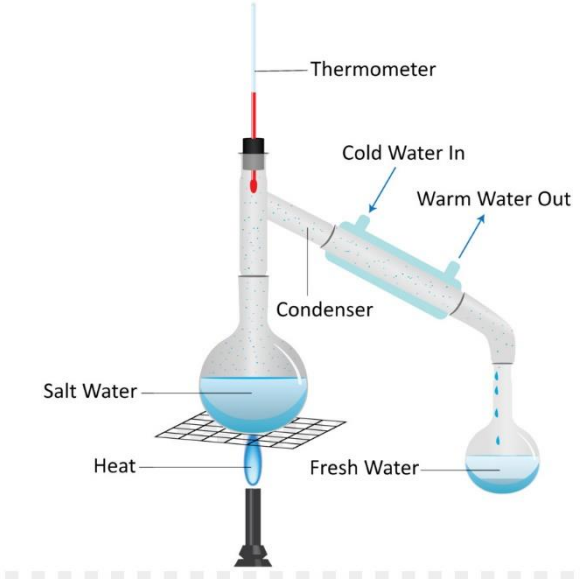
-Allow the solution to cool slowly, preferably to room temperature, to allow crystals to form.

-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 :

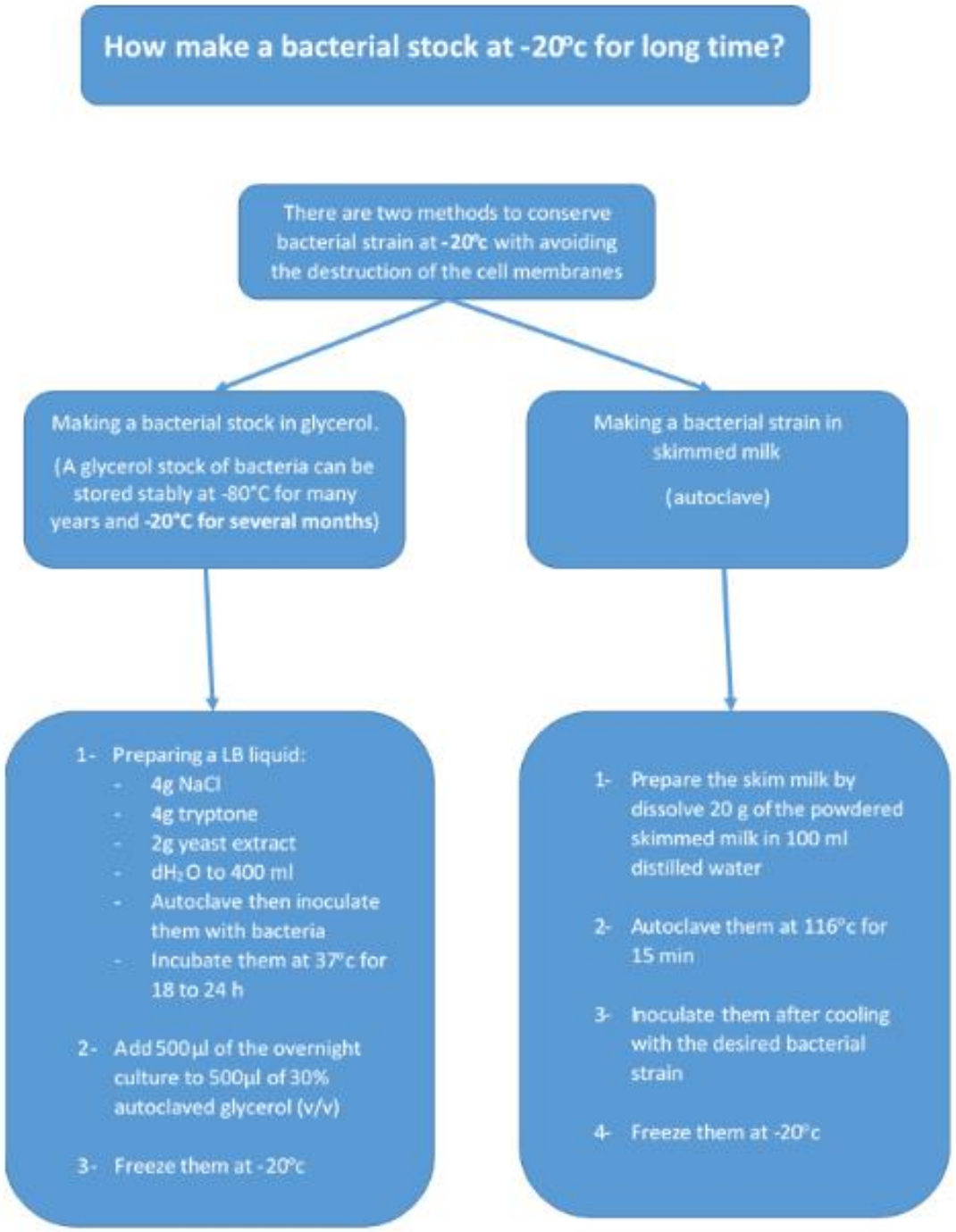
Extraction of salicylic acid: It is generally extracted first because it is the main compound in the reaction mixture of the synthesis of aspirin.

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.

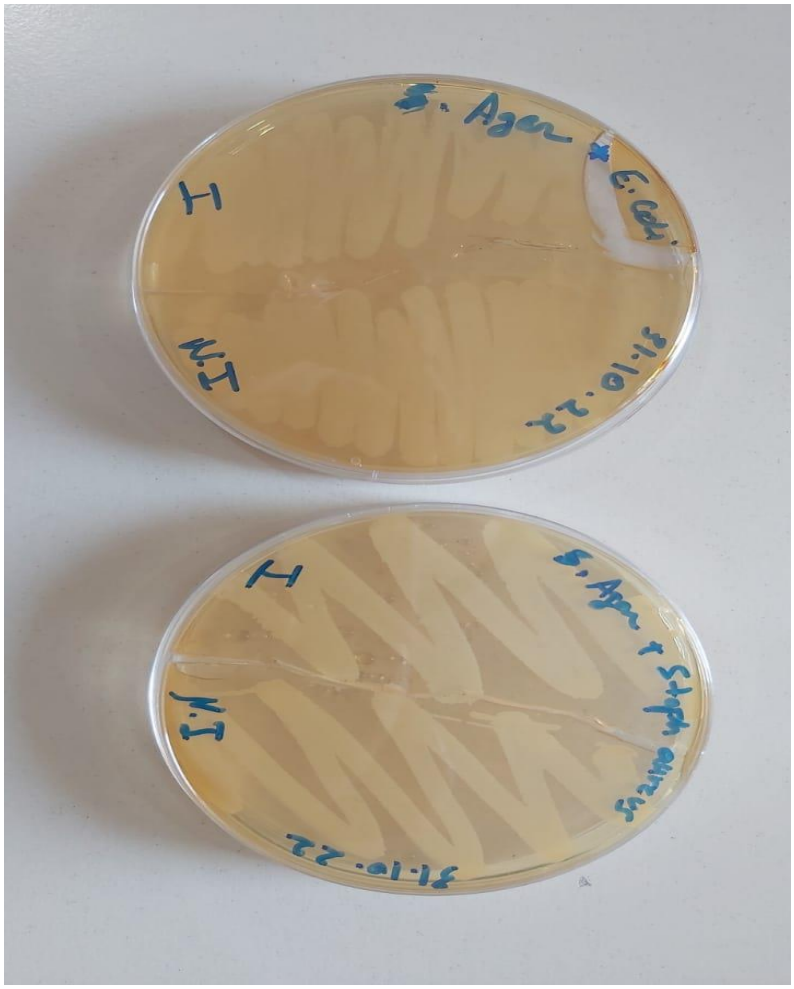
18 Annex: The most important solutions in biology lab

18.1 Making bacterial stock solution:27-10-2022



18.2 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 inoculate d in skim milk before freezing at -20°c



18.2.1 Solution preparation

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).

H_2SO_4(1M):

-Fill the flask half way with distilled water

Add 56 ml of H_2SO_4

-add water to reach the 1L mark

-Finally mix it to obtain H_2SO_4 solution (1M).

NaCl 0.9%:

-We put 0.9g NaCl in 100 ml distilled water

-Put it in Autoclave for microbial usage.

Bleach 1%:

-We took 1ml of bleach solution

-Put it in 100ml water graduated cylinder

-Mix it to obtain Bleach solution 1%.

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.

Standard agar:

-Measure 5.55g of standard agar 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.

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.

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.

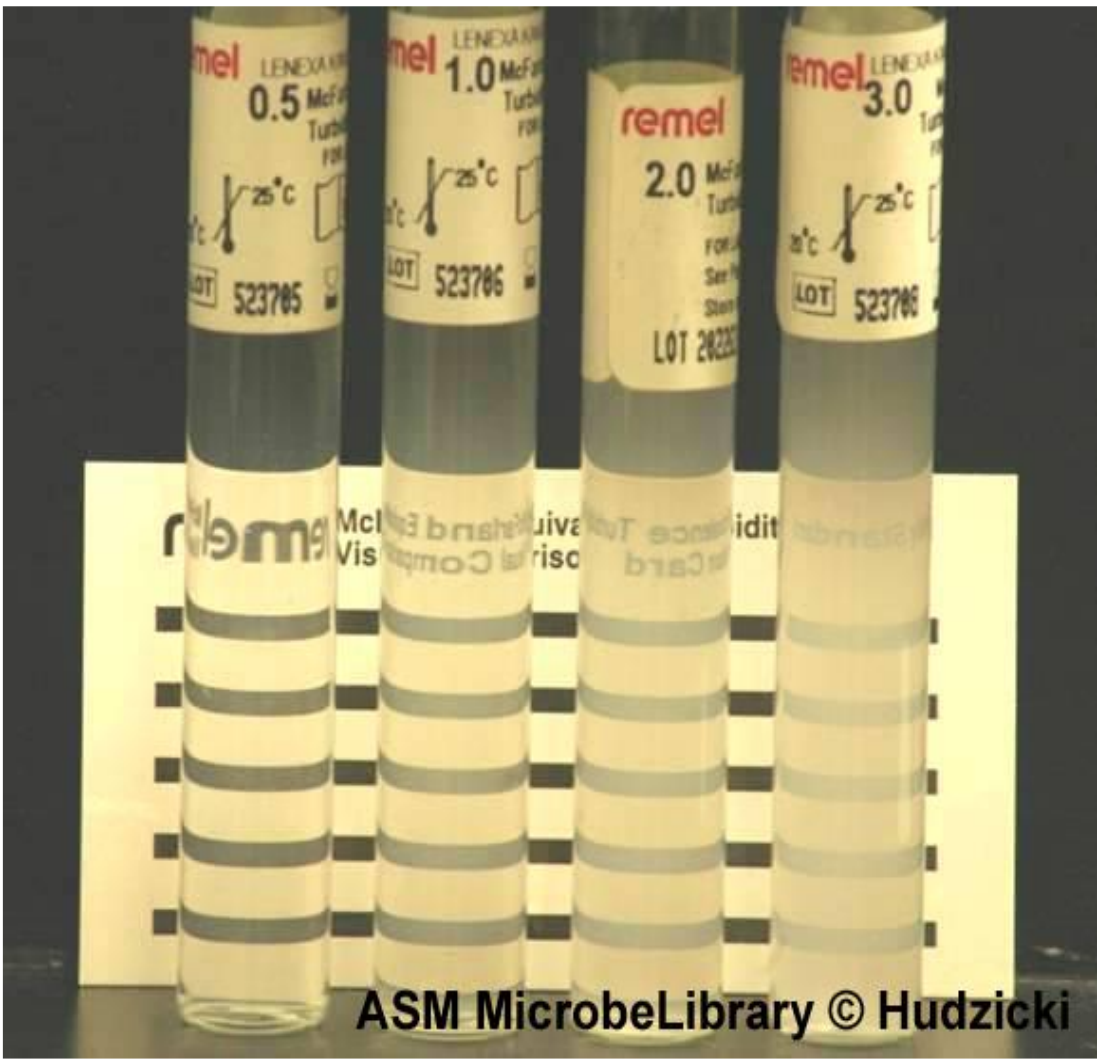
18.3 Preparation of the turbidity calibration 0.5 McFarland:(40)

1. we added 0.5 mL of a 0.048 mol/L solution of $BaCl_2$ (1.175% w/v $BaCl_2$ $2H_2O$) to 99.5 mL of a 0.18 mol/L solution (0.36 N) of H_2SO_4 (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)



18.4 *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$

18.5 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**	

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2. cycles T text provides general information S assumes no liability for the information given being complete or correct D to varying update, Text SCDM up to DDTR in the. Topic: Bayer AG [Internet]. Statista. [cited 2022 Dec 8]. Available from: https://www.statista.com/topics/4292/bayer-ag/
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GREEN CHEMISTRY

MEGBI REPORT 2024

Chemicals Production for Aspirin Production Plant

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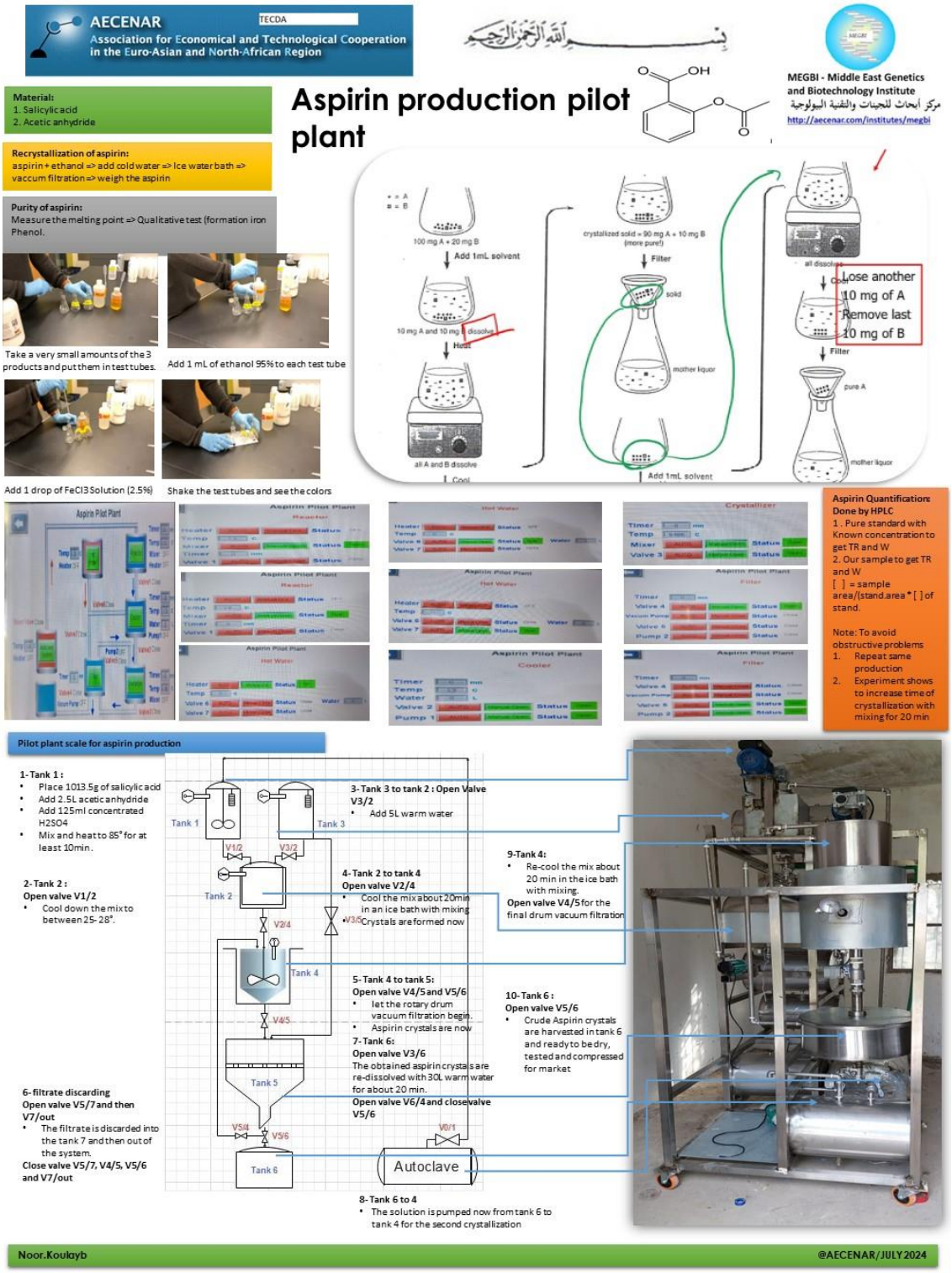
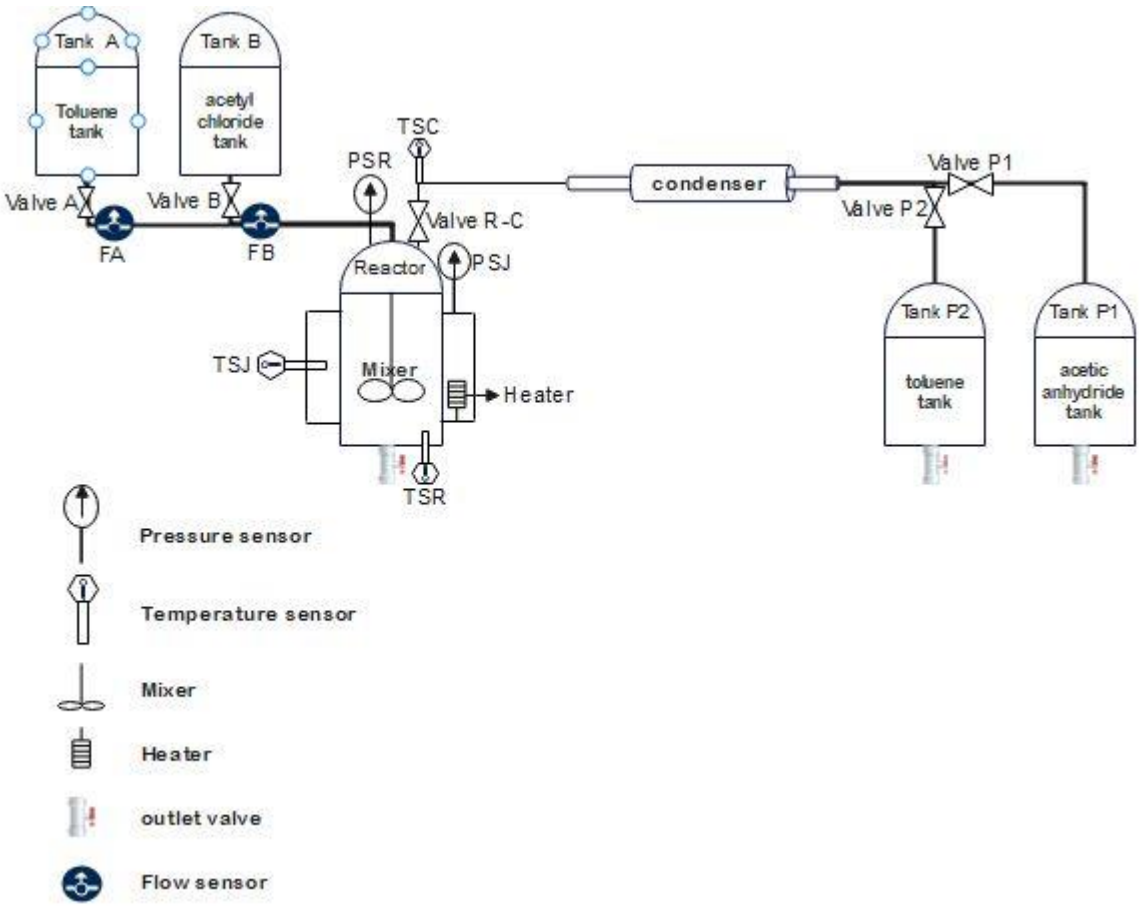
Last Update: 24.03.2025 15:17

19 Summary

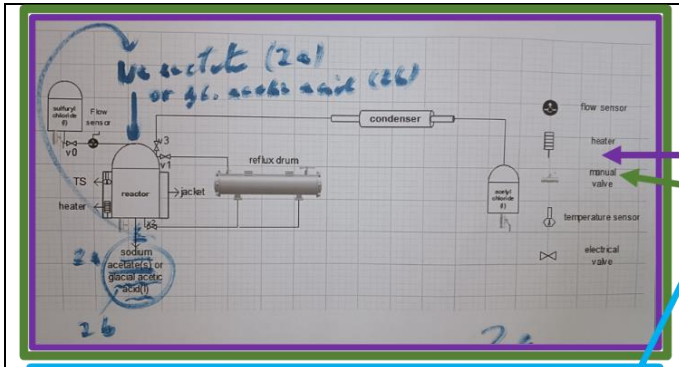
19.1 Aspirin Production Plant

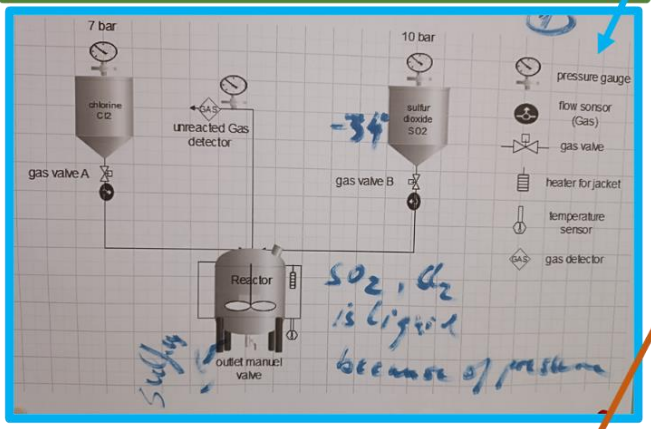
To produce Aspirin, the two raw materials salicylic acid and acetic anhydride are needed. In 2024 all pathways where worked out to produce acetic anhydride from basic, available materials. Also an acetic anhydride pilot plant was designed and the devices were realized.

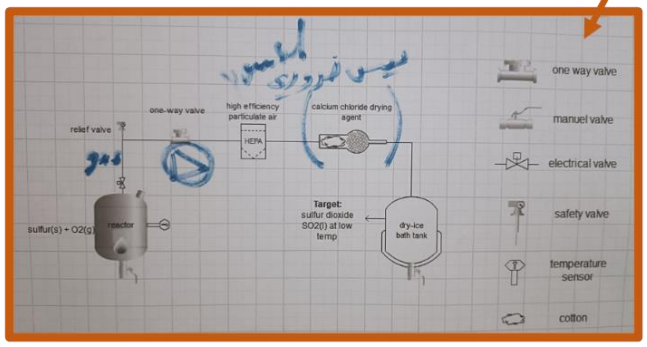


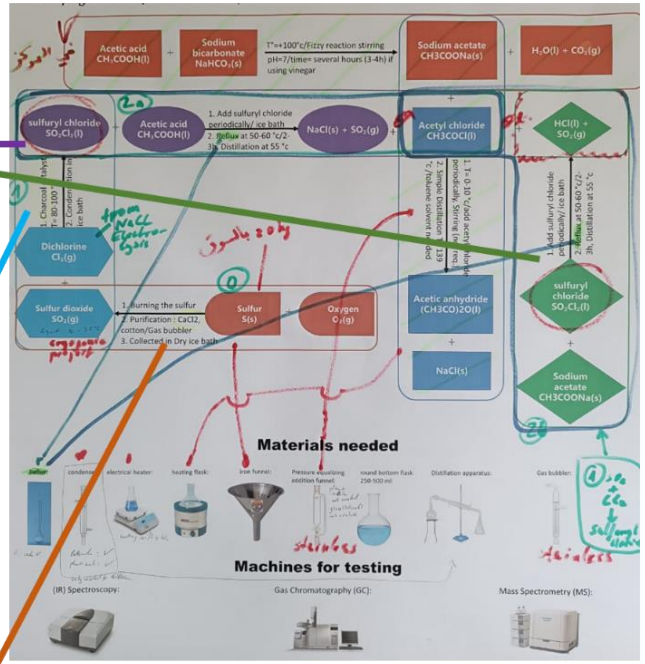

Tank A
Toluene tank
Tank B
acetyl chloride tank
Valve A
Valve B
FA
FB
TSC
PSR
Valve R-C
Reactor
PSJ
TSJ
Mixer
Heater
TSR
condenser
Valve P1
Valve P2
Tank P2
toluene tank
Tank P1
acetic anhydride tank
Pressure sensor
Temperature sensor
Mixer
Heater
outlet valve
Flow sensor

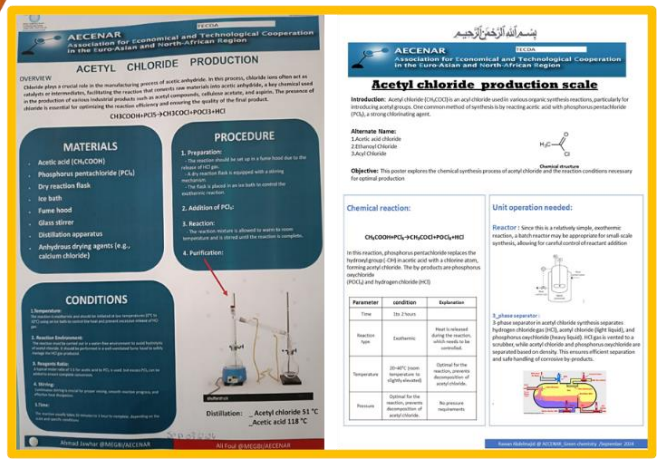
19.2 Overview of reactions to produce acetic anhydride









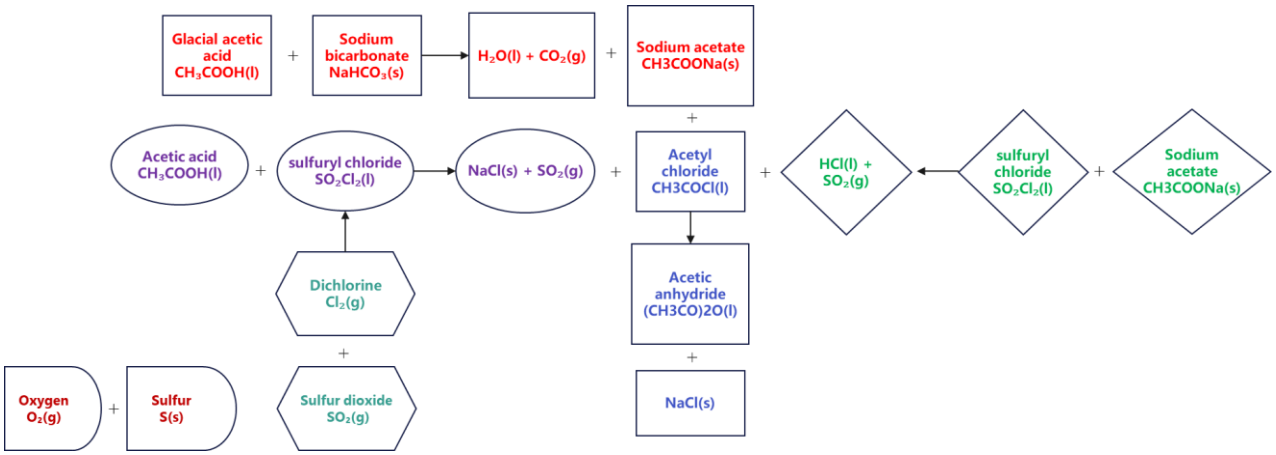


Overview reaction [pptx file:

Overview reactions.pptx

19.3 On aecenar.com site

19.4 Path to produce acetic anhydride (acetyl chloride with sulfur path)



General flow chart [pptx file]:	Drawing2.pptx

For aspirin we need as raw material acetic anhydride. So the goal of is to produce Acetic Anhydride [$(CH_3CO)_2O$] according to the following reaction:

Sodium Acetate + Acetyl Chloride → Acetic Anhydride + Sodium Chloride

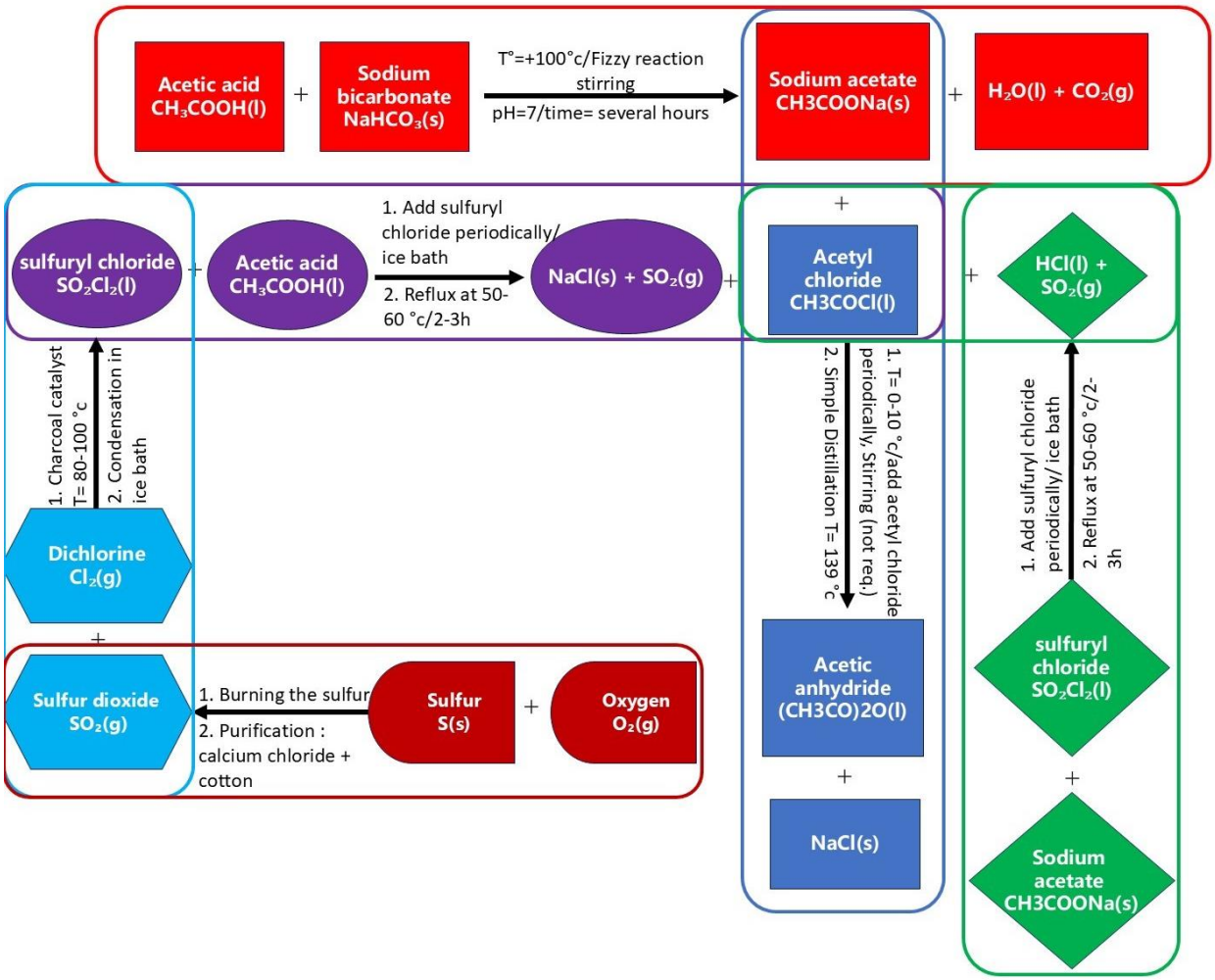
$CH_3COONa_{(s)} + CH_3COCl_{(l)} \rightarrow (CH_3CO)_2O_{(l)} + NaCl_{(s)}$

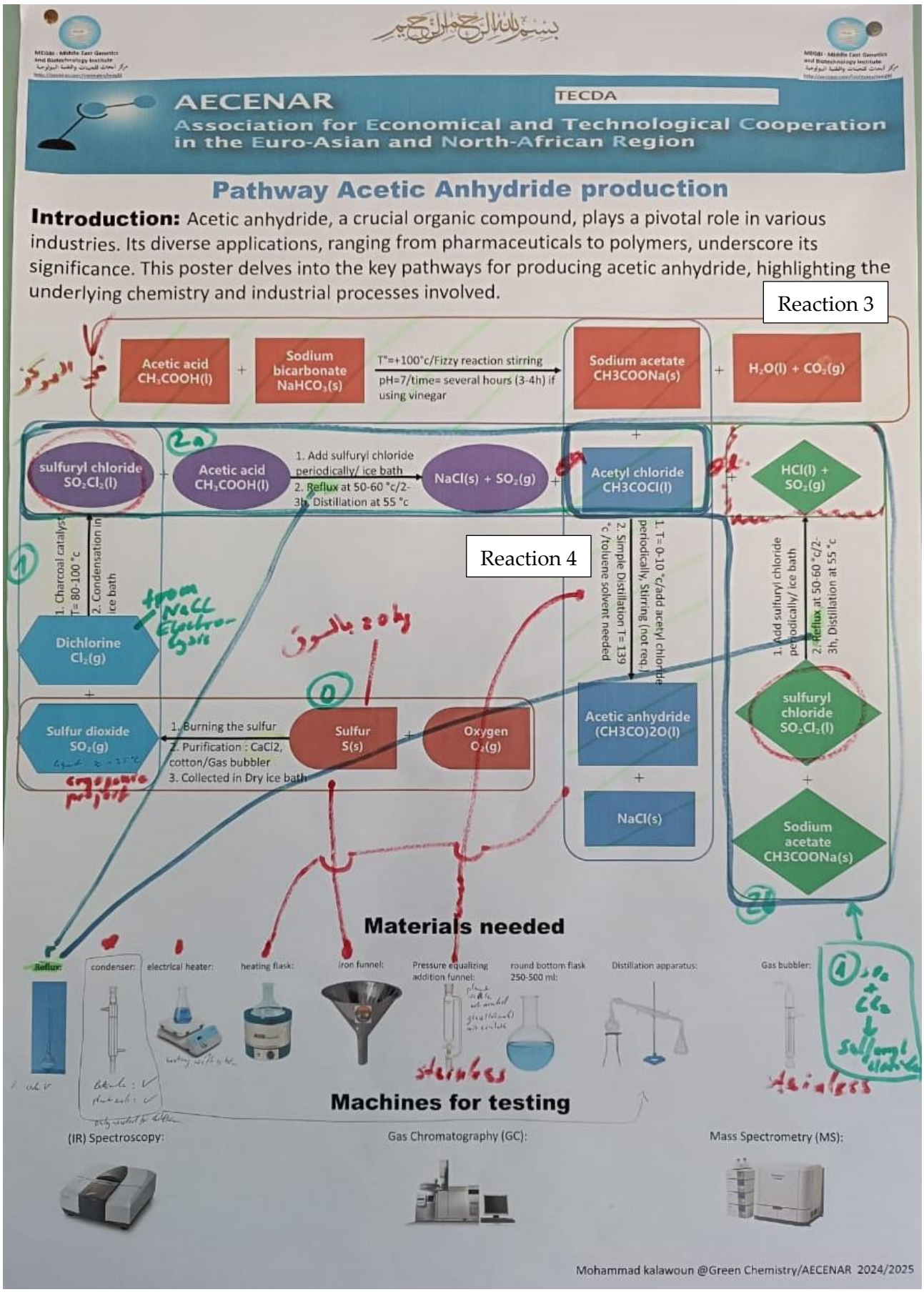
This reaction occurs under the following conditions:

1) T = 0-10℃, Add Acetyl Chloride periodically, Stirring (not req.)
2) Simple distillation at T = 139℃ / Toluene solvent needed

To achieve this reaction, Sodium Acetate and Acetyl Chloride must be obtained.

And therefore we need to produce Acetyl Chloride.


Acetic acid $CH_3COOH(l)$
Sodium bicarbonate $NaHCO_3(s)$
T°=+100°c/Fizzy reaction
stirring
pH=7/time= several hours
Sodium acetate CH3COONa(s)
$H_2O(l) + CO_2(g)$
sulfuryl chloride $SO_2Cl_2(l)$
Acetic acid $CH_3COOH(l)$
1. Add sulfuryl chloride periodically/ ice bath
2. Reflux at 50-60 °c/2-3h
$NaCl(s) + SO_2(g)$
Acetyl chloride CH3COCl(l)
$HCl(l) + SO_2(g)$
1. Charcoal catalyst T= 80-100 °c
2. Condensation in ice bath
Dichlorine $Cl_2(g)$
2. Simple Distillation T= 139 °c
1. T= 0-10 °c/add acetyl chloride periodically, Stirring (not req.)
1. Add sulfuryl chloride periodically/ ice bath
2. Reflux at 50-60 °c/2-3h
Sulfur dioxide $SO_2(g)$
1. Burning the sulfur
2. Purification : calcium chloride + cotton
Sulfur S(s)
Oxygen $O_2(g)$
Acetic anhydride $(CH3CO)2O(l)$
NaCl(s)
sulfuryl chloride $SO_2Cl_2(l)$
Sodium acetate CH3COONa(s)


بسم الله الرحمن الرحيم
AECENAR
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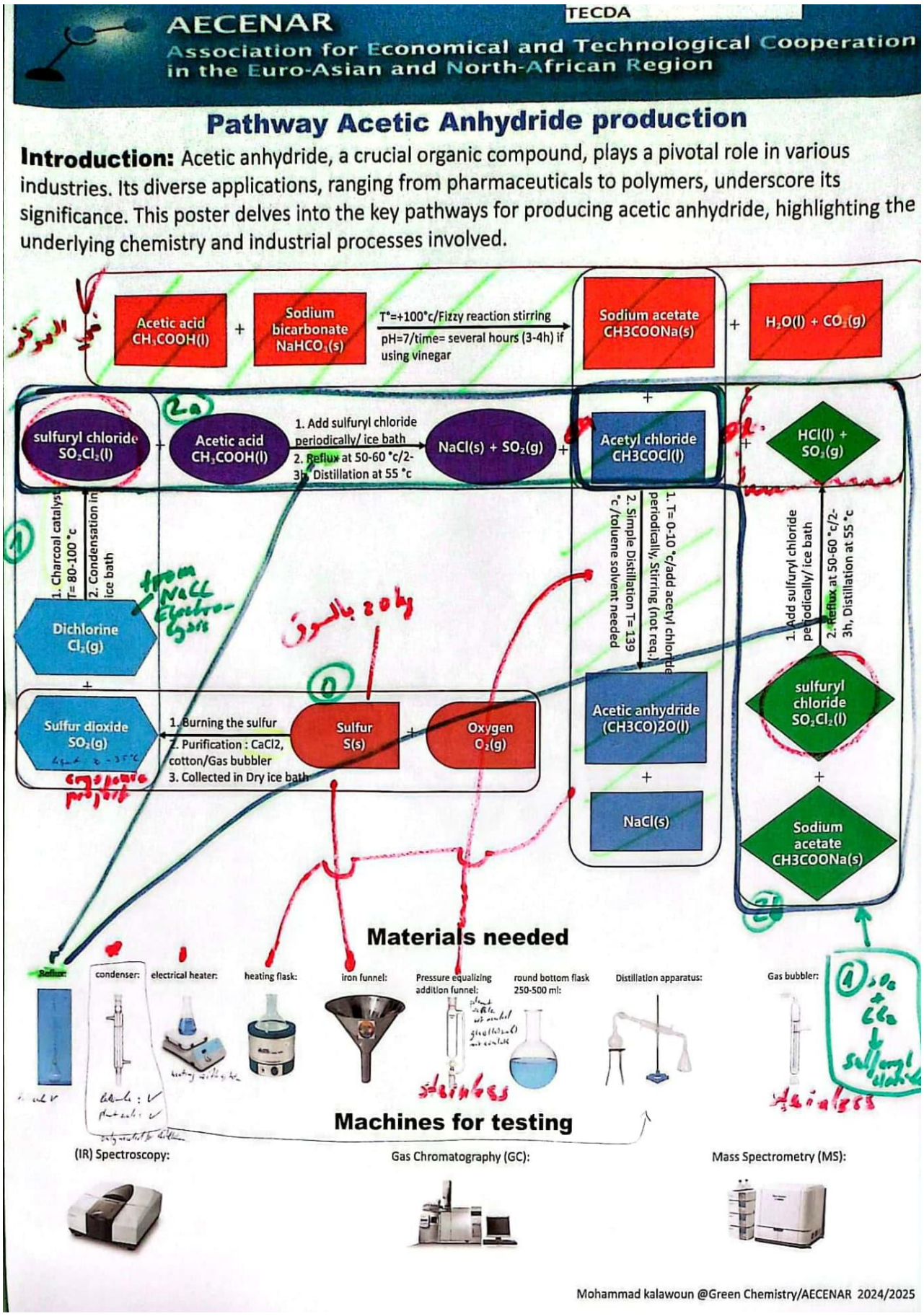
Association for Economical and Technological Cooperation in the Euro-Asian and North-African Region
Pathway Acetic Anhydride production
Introduction: Acetic anhydride, a crucial organic compound, plays a pivotal role in various industries. Its diverse applications, ranging from pharmaceuticals to polymers, underscore its significance. This poster delves into the key pathways for producing acetic anhydride, highlighting the underlying chemistry and industrial processes involved.
Reaction 3
Acetic acid CH3COOH(l)
Sodium bicarbonate NaHCO3(s)
T°=+100°c/Fizzy reaction stirring
pH=7/time= several hours (3-4h) if using vinegar
Sodium acetate CH3COONa(s)
H2O(l) + CO2(g)
sulfuryl chloride SO2Cl2(l)
Acetic acid CH3COOH(l)
1. Add sulfuryl chloride periodically/ ice bath
2. Reflux at 50-60 °c/2-3h, Distillation at 55 °c
NaCl(s) + SO2(g)
Acetyl chloride CH3COCl(l)
HCl(l) + SO2(g)
Reaction 4
1. Charcoal catalyst T= 80-100 °c
2. Condensation in ice bath
Dichlorine Cl2(g)
1. T= 0-10 °c/add acetyl chloride periodically, Stirring (not req.)
2. Simple Distillation T= 139 °c /toluene solvent needed
1. Add sulfuryl chloride periodically/ ice bath
2. Reflux at 50-60 °c/2-3h, Distillation at 55 °c
Sulfur dioxide SO2(g)
1. Burning the sulfur
2. Purification : CaCl2, cotton/Gas bubbler
3. Collected in Dry ice bath
Sulfur S(s)
Oxygen O2(g)
Acetic anhydride (CH3CO)2O(l)
NaCl(s)
sulfuryl chloride SO2Cl2(l)
Sodium acetate CH3COONa(s)
Materials needed
Reflux:
condenser:
electrical heater:
heating flask:
iron funnel:
Pressure equalizing addition funnel:
round bottom flask 250-500 ml:
Distillation apparatus:
Gas bubbler:
Machines for testing
(IR) Spectroscopy:
Gas Chromatography (GC):
Mass Spectrometry (MS):
Mohammad kalawoun @Green Chemistry/AECENAR 2024/2025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1. Burning the sulfur
2. Purification : CaCl2, cotton/Gas bubbler
3. Collected in Dry ice bath
Sulfur S(s)
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1. T= 0-10 °c/add acetyl chloride periodically, Stirring (not req.)
2. Simple Distillation T= 139 °c /toluene solvent needed
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NaCl(s)
1. Add sulfuryl chloride periodically/ ice bath
2. Reflux at 50-60 °c/2-3h, Distillation at 55 °c
sulfuryl chloride SO2Cl2(l)
Sodium acetate CH3COONa(s)
Materials needed
Reflux:
condenser:
electrical heater:
heating flask:
iron funnel:
Pressure equalizing addition funnel:
round bottom flask 250-500 ml:
Distillation apparatus:
Gas bubbler:
Machines for testing
(IR) Spectroscopy:
Gas Chromatography (GC):
Mass Spectrometry (MS):
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Dichlorine can we get from NaCl electrolysis

19.4.1 Dichlorine (Cl_2) production

Dichlorine (Cl_2) can be obtained from the NaCl electrolysis

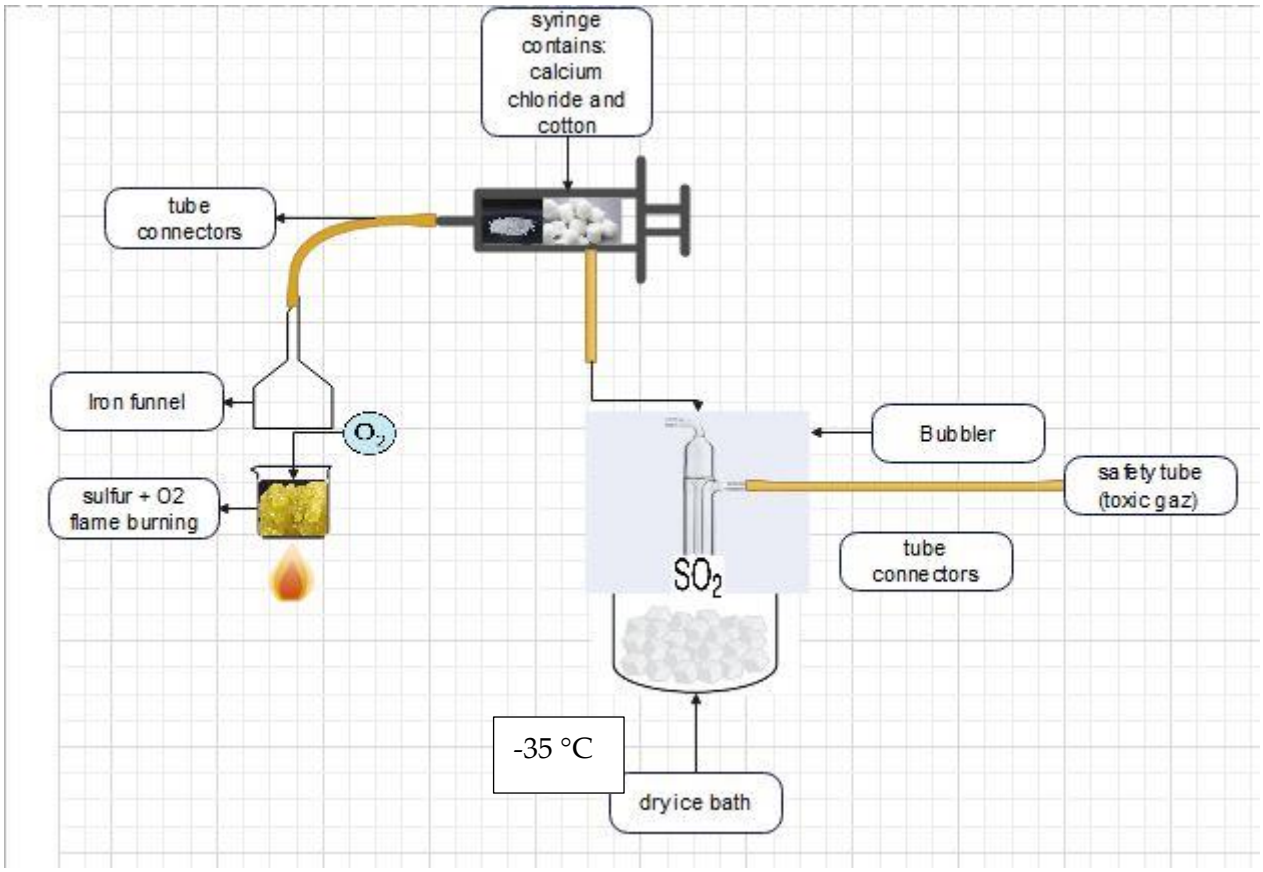
19.4.2 Sulfur dioxide production (reaction 0)

Sulfur Dioxide can produced by the following reaction:

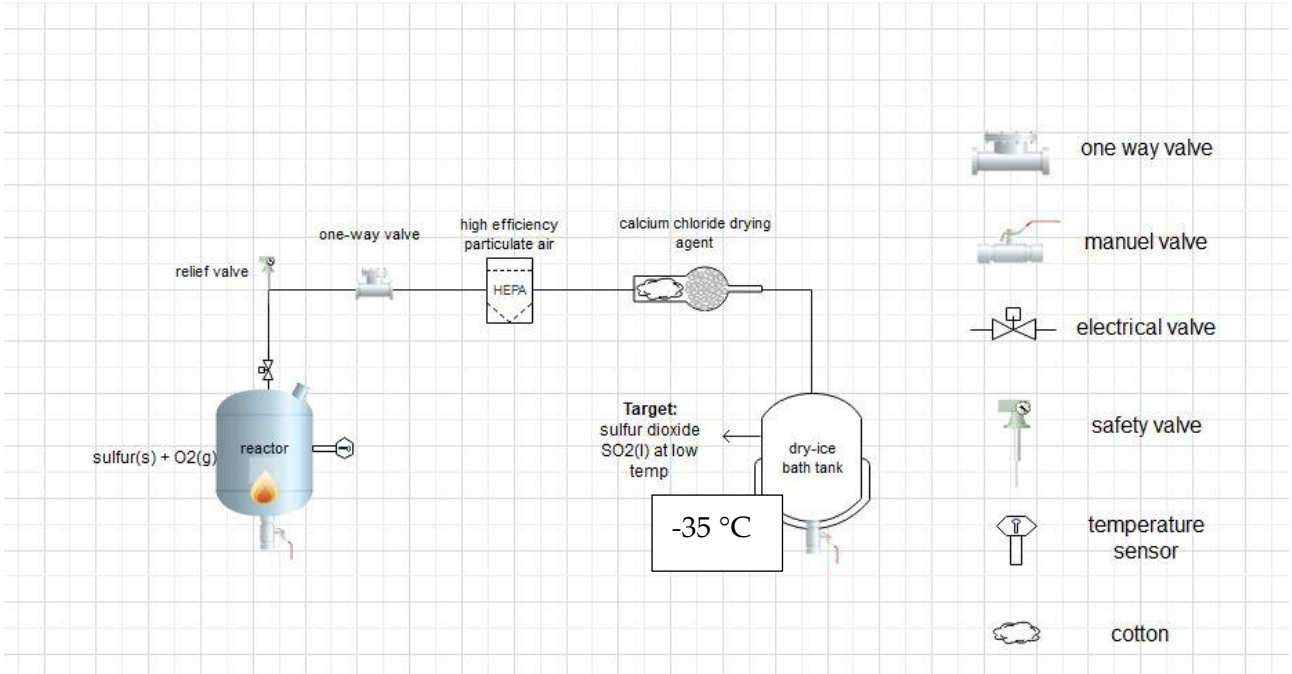
$$\text{Sulfur} + \text{Oxygen} \rightarrow \text{Sulfur Dioxide}$$

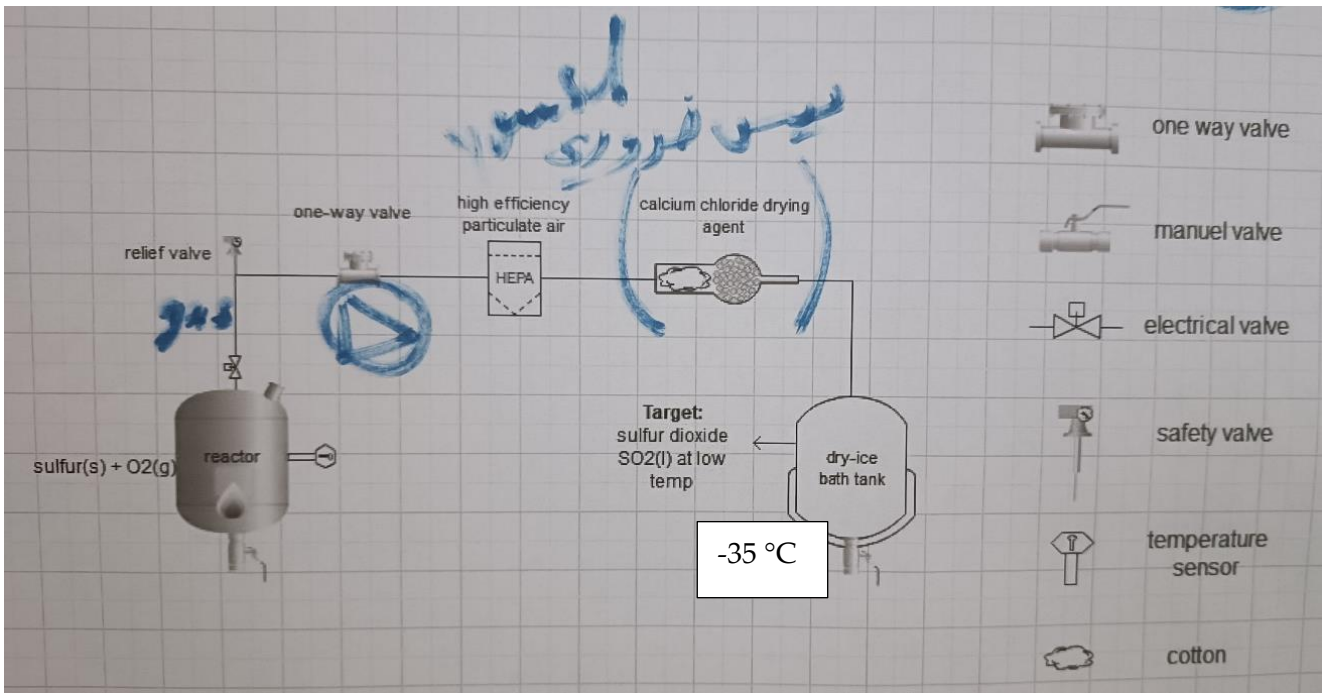
$$S_{(s)} + O_{2(g)} \rightarrow SO_{2(g)}$$

19.4.2.1 Lab Scale



19.4.2.2 Pilot Plant Scale





This reaction occurs under the following conditions:

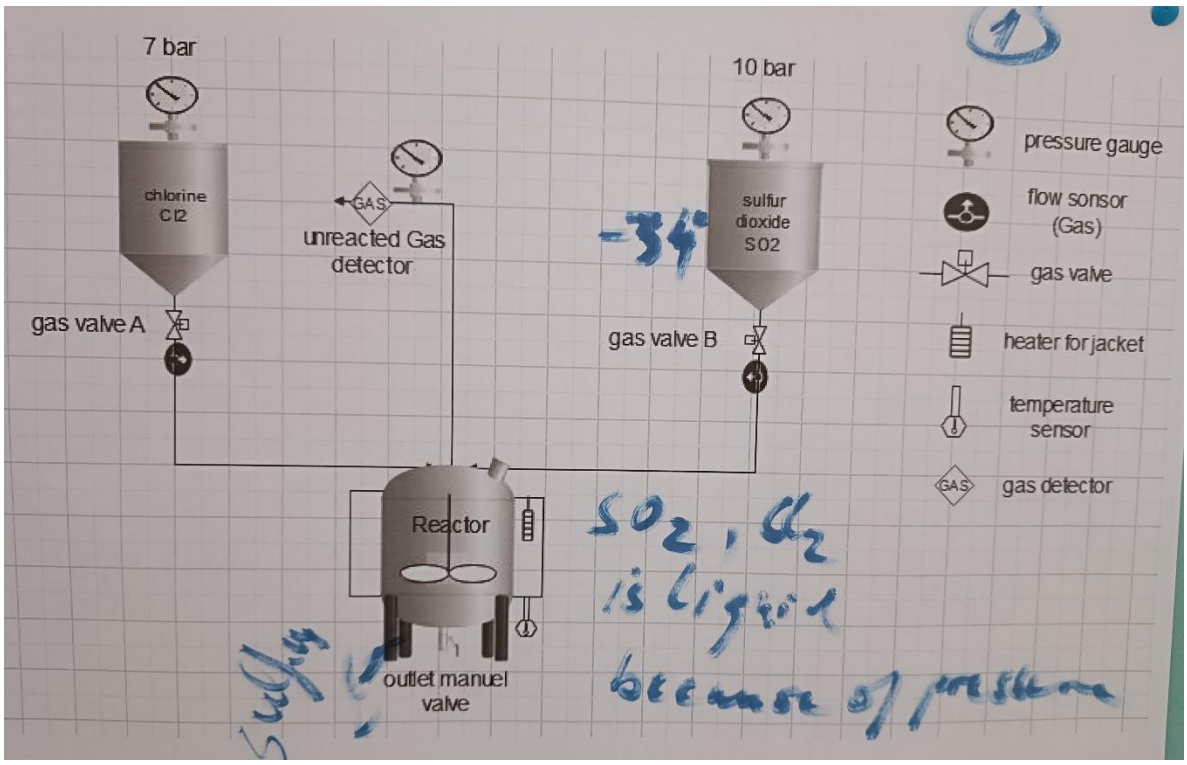
1) Burning the Sulfur
2) Purification with $CaCl_2$ cotton / Gas bubbler
3) Collected in Dry ice bath

Note: SO_2 liquefied at -35℃ [Cryogenic project]

19.4.3 Sulfuryl chloride ($SO_2Cl_{2(l)}$) production (reaction 1)

Sulfur dioxide + Dichlorine → Sulfuryl Chloride

$$SO_{2(g)} + Cl_{2(g)} \rightarrow SO_2Cl_{2(l)}$$



This reaction occurs under the following conditions:

1) Charcoal catalyst T = 80-100°C
2) Condensation in an ice bath

19.4.4 Acetyl chloride production (reaction 2a and 2b)

We can obtain Acetyl Chloride [CH_3COCl] can be obtained in the following two ways:

- **1st way (reaction 2a)**, Through the following reaction:

Sulfuryl Chloride + Acetic Acid → Sodium Chloride + Acetyl Chloride

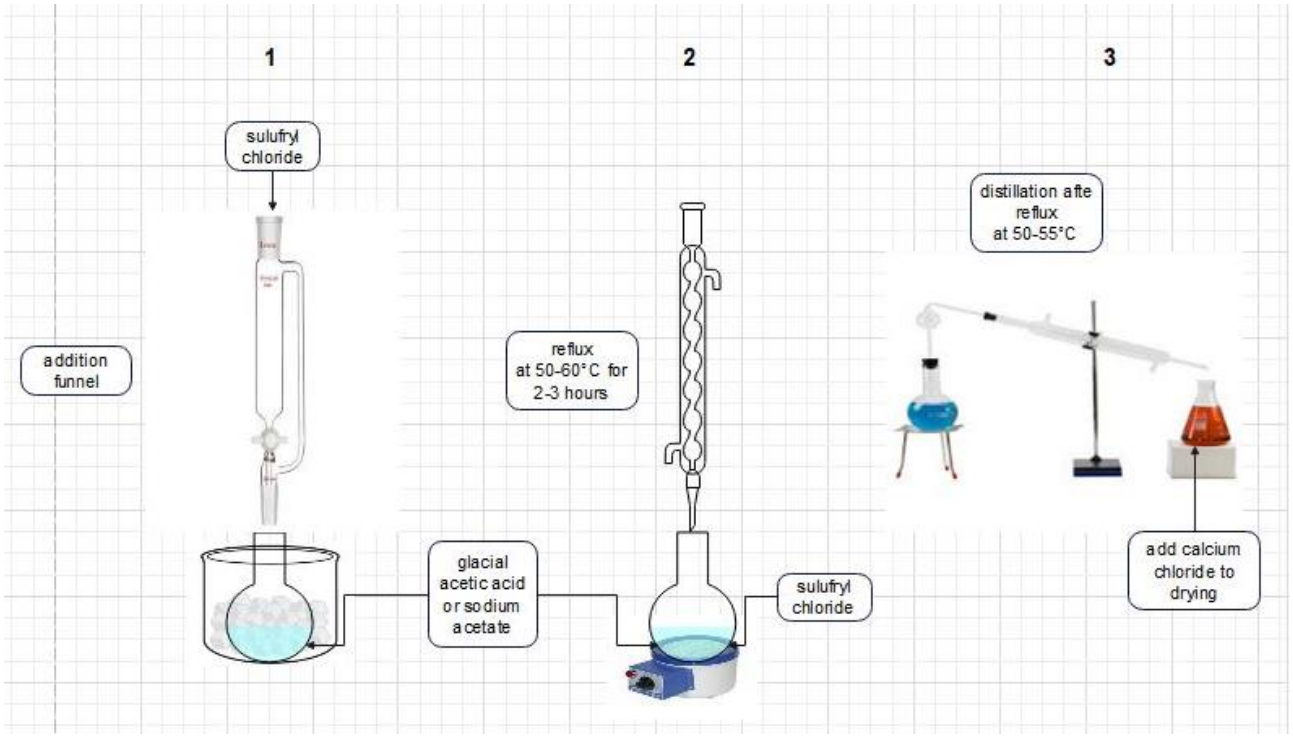
$$SO_2Cl_{2(l)} + CH_3COOH_{(l)} \rightarrow SO_{2(g)} + CH_3COCl_{(l)}$$

- **2nd way (reaction 2b)**, Through the following reaction:

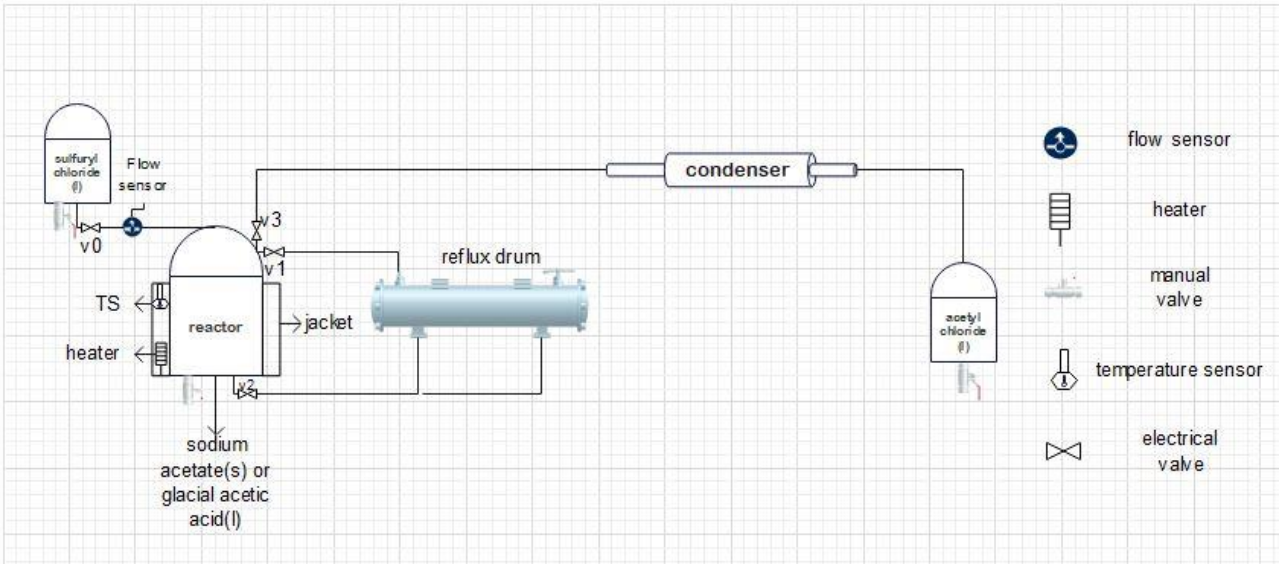
Sodium Acetate + Sulfuryl Chloride → Hydrochloric Acid + Acetyl Chloride + Sulfur Dioxide

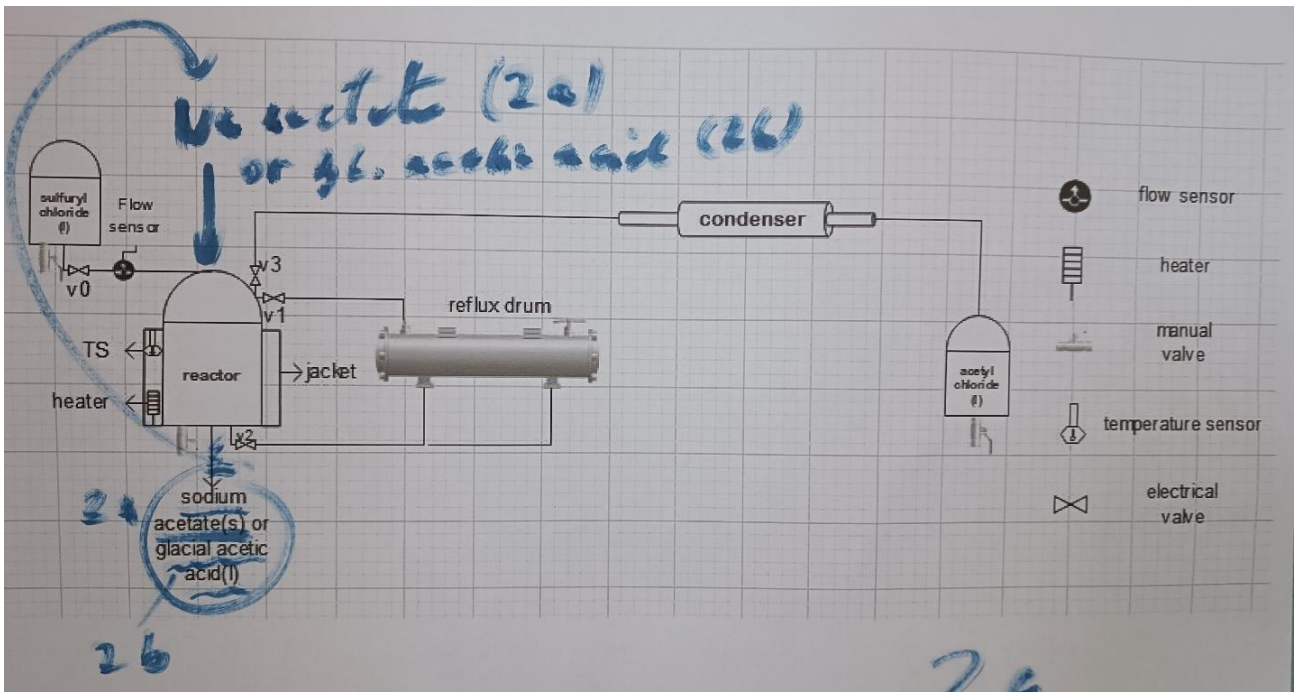
$$CH_3COONa_{(s)} + SO_2Cl_{2(l)} \rightarrow HCl_{(l)} + CH_3COCl_{(l)} + SO_{2(g)}$$

19.4.4.1 Lab Scale



19.4.4.2 Pilot Plant Scale

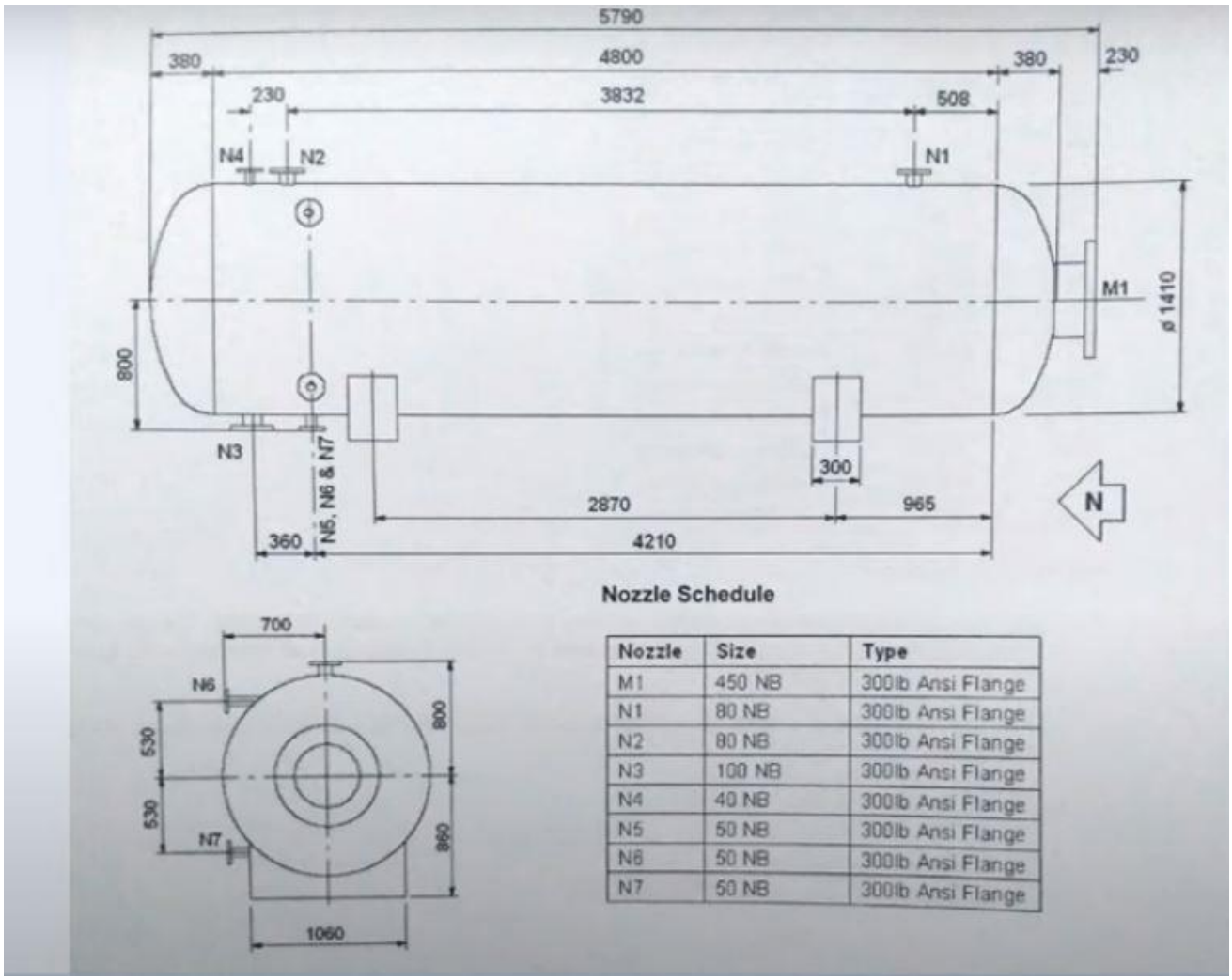




This reaction occurs under the following conditions:

1) Add Sulfryl Chloride periodically / ice bath
2) Reflux at 50-60°C, time: 2-3 hrs with distillation at 55°C

Reflux Drum:



Nozzle Schedule

Nozzle	Size	Type
M1	450 NB	300lb Ansi Flange
N1	80 NB	300lb Ansi Flange
N2	80 NB	300lb Ansi Flange
N3	100 NB	300lb Ansi Flange
N4	40 NB	300lb Ansi Flange
N5	50 NB	300lb Ansi Flange
N6	50 NB	300lb Ansi Flange
N7	50 NB	300lb Ansi Flange

19.4.5 Production of sodium acetate (reaction 3)

- Production of Sodium Acetate [CH_3COONa]:

We can obtain Sodium Acetate [CH_3COONa] through the following reaction:

Acetic Acid + Sodium Bicarbonate → Sodium Acetate + Water + Carbon dioxide

$CH_3COOH_{(l)} + NaHCO_{3(s)} \rightarrow CH_3COONa_{(s)} + H_2O_{(l)} + CO_{2(g)}$

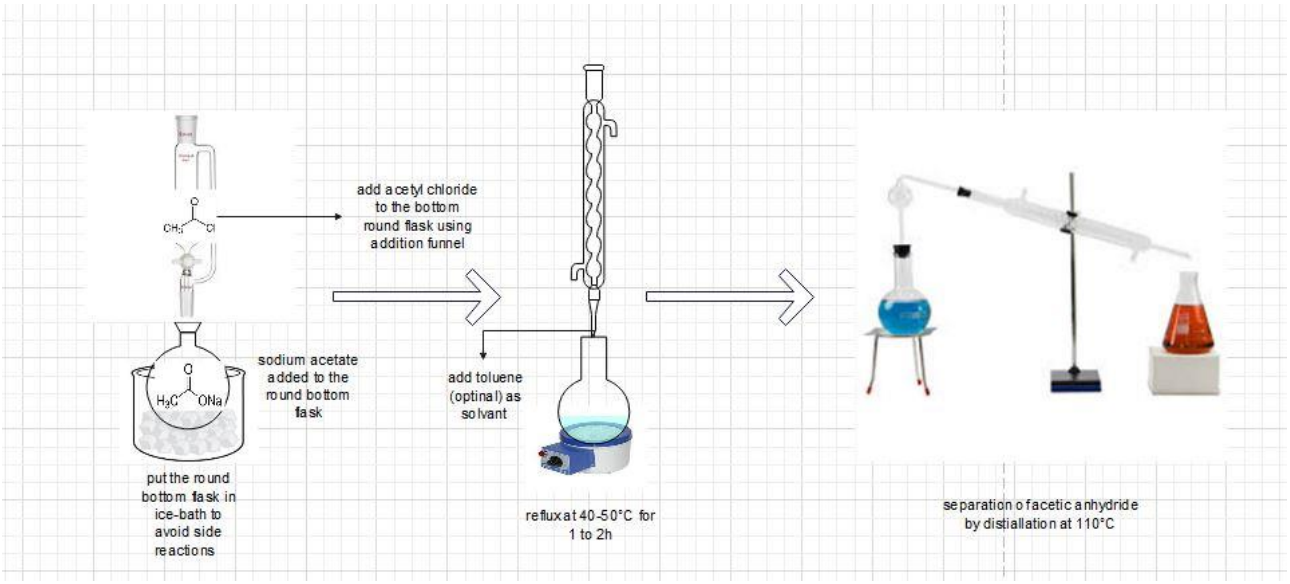
This reaction occurs under the following conditions:

1) T = +100°C, Fizzy reaction Stirring
2) pH=7 / time : several hours(3-4 hrs) if using vinegar

Acetic Acid and Sodium Bicarbonate must be obtained to achieve this reaction, or they are available.

19.4.6 Acetic Anhydride Production (reaction 4)

19.4.6.1 Lab Scale



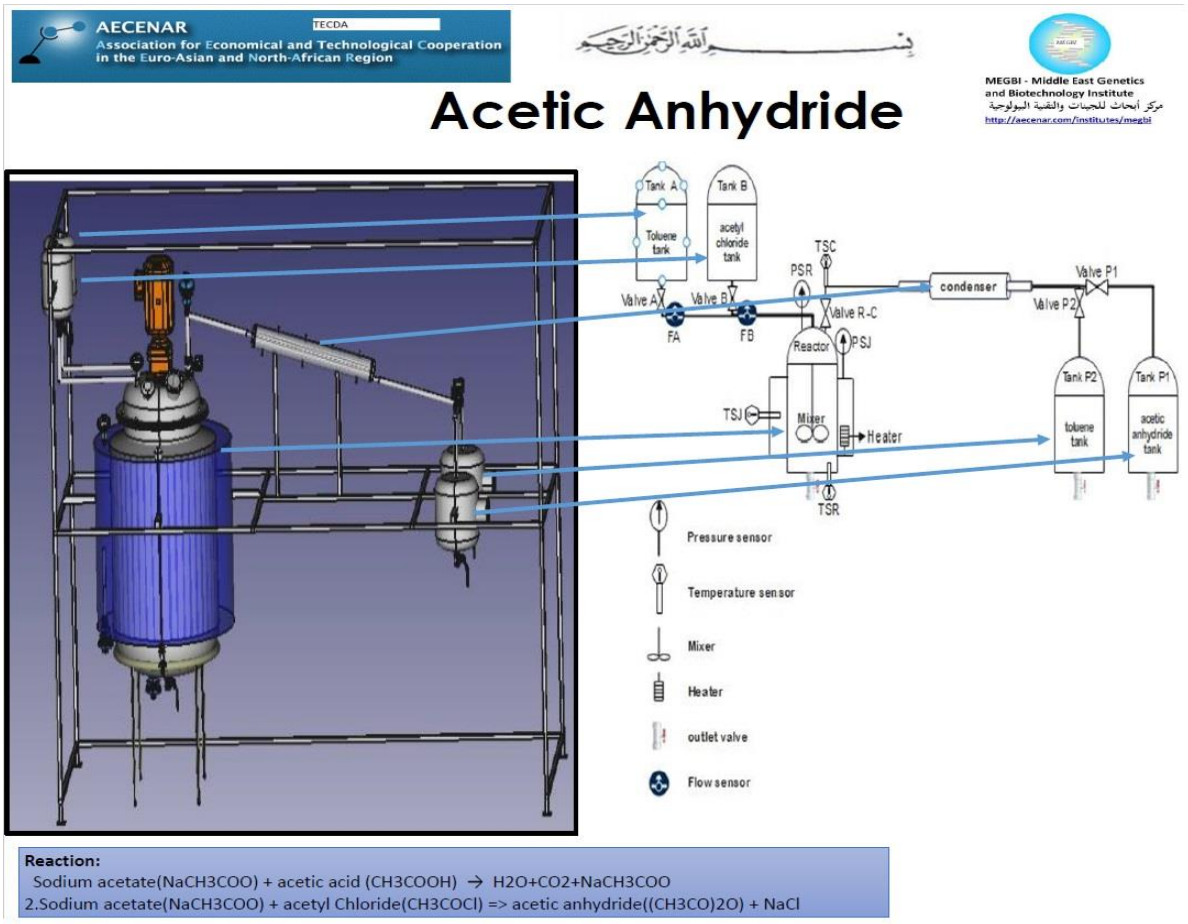
Sodium Acetate + Acetyl Chloride → Acetic Anhydride + Sodium Chloride

$$CH_3COONa_{(s)} + CH_3COCl_{(l)} \rightarrow (CH_3CO)_2O_{(l)} + NaCl_{(s)}$$

This reaction occurs under the following conditions:

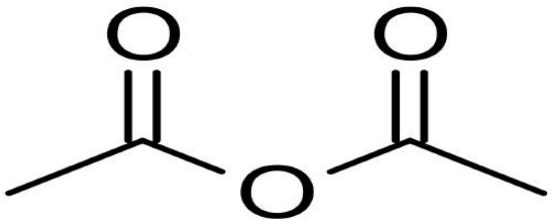
1) T = 0-10°C, Add Acetyl Chloride periodically, Stirring (not req.)
2) Simple distillation at T = 139°C / Toluene solvent needed

19.4.6.2 Pilot plant scale



Material:
- Vinegar
- Sodium bicarbonate
- Acetyl chloride (C2H3ClO)

Safety:
1.Toluene: Sweet aroma,
2. Acetyl chloride: Corrosive, flammable
3.Acetic anhydride: Highly corrosive, inhalation damages lungs,



Procedure:
1. Mixer activation
2. Reagent addition for reaction 1"vinger + Na + C2H3ClO"
3. Heat to 120°C
4. Cool Down to 50-80°C
5. Add 5L of toluene as "solvent"
6. Distillation
7. Test purity and yield of acetic anhydride.

Requirements:
1. Physical: Pipes, tanks, and autoclave must withstand high temperatures (T = 300°C) and pressures (P = 260 bar).
2. Chemical: - Tanks, autoclaves, pipes, valves shall be withstand corrosion with acetic anhydride, toluene , acetic acetate and acetyl chloride
3. Mechanical:
system: stain less steel 316, close
Valve: close compound
Sensor: road data from system

Noor.Koulayb @AECENAR/JULY 2024

19.5 Acetyl chloride with phosphor path (lab scale and pilot plant scale)

Acetic Acid + Phosphorus pentachloride → Acetyl Chloride + Phosphoryl Chloride + Hydrogen Chloride

$$CH_3COOH_{(l)} + PCl_{5(s)} \rightarrow CH_3COCl_{(l)} + POCl_{3(l)} + HCl_{(g)}$$

19.5.1 Procedure

19.5.1.1 Preparation:

- The reaction should be set up in a fume hood due to the release of HCl gas.
- A dry reaction flask is equipped with a stirring mechanism.
- The flask is placed in an ice bath to control the exothermic reaction.

1) Addition of PCl5
2) Reaction: The reaction mixture is allowed to warm to room temperature and is stirred until the reaction is complete.
3) Purification: By distillation, Acetyl chloride (at T = 51°C) and Acetic Acid (at T = 118°C).

19.5.1.2 Reaction Conditions

This reaction occurs under the following conditions:

1) Temperature: The reaction is exothermic and should be initiated at low temperature (0°C to 10°C) using an ice bath to control the heat and prevent excessive release of HCl gas
2) Reaction environment: To avoid hydrolysis of acetyl chloride, the reaction must be carried out in a water-free environment. To safely manage the HCl gas produced, it should be performed in a well-ventilated fume hood.
3) Reagents ratio: A typical molar ratio of 1:1 for Acetic Acid to PCl_5 is used, but excess PCl5 can be added to ensure complete conversion.
4) Stirring: Continuous stirring is crucial for proper mixing, smooth reaction progress, and effective heat dissipation.
5) Time: The reaction usually takes 30 minutes to 1 hour to complete, depending on the scale and specific conditions.

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ACETYL CHLORIDE PRODUCTION

OVERVIEW

Chloride plays a crucial role in the manufacturing process of acetic anhydride. In this process, chloride ions often act as catalysts or intermediates, facilitating the reaction that converts raw materials into acetic anhydride, a key chemical used in the production of various industrial products such as acetyl compounds, cellulose acetate, and aspirin. The presence of chloride is essential for optimizing the reaction efficiency and ensuring the quality of the final product.

$$CH_3COOH + PCl_5 \rightarrow CH_3COCl + POCl_3 + HCl$$

MATERIALS

- Acetic acid (CH_3COOH)
- Phosphorus pentachloride (PCl_5)
- Dry reaction flask
- Ice bath
- Fume hood
- Glass stirrer
- Distillation apparatus
- Anhydrous drying agents (e.g., calcium chloride)

PROCEDURE

1. Preparation:
- The reaction should be set up in a fume hood due to the release of HCl gas.
- A dry reaction flask is equipped with a stirring mechanism.
- The flask is placed in an ice bath to control the exothermic reaction.

2. Addition of PCl_5:

3. Reaction:
- The reaction mixture is allowed to warm to room temperature and is stirred until the reaction is complete.

4. Purification:

CONDITIONS

1.Temperature:
The reaction is exothermic and should be initiated at low temperatures (0°C to 10°C) using an ice bath to control the heat and prevent excessive release of HCl gas.

2. Reaction Environment:
The reaction must be carried out in a water-free environment to avoid hydrolysis of acetyl chloride. It should be performed in a well-ventilated fume hood to safely manage the HCl gas produced.

3. Reagents Ratio:
A typical molar ratio of 1:1 for acetic acid to PCl_5 is used, but excess PCl_5 can be added to ensure complete conversion.

4. Stirring:
Continuous stirring is crucial for proper mixing, smooth reaction progress, and effective heat dissipation.

5.Time:
The reaction usually takes 30 minutes to 1 hour to complete, depending on the scale and specific conditions

Distillation: _ Acetyl chloride 51 °C
_Acetic acid 118 °C

Sep 03/2024

Ahmad Jawhar @MEGBI/AECENAR
Ali Foul @MEGBI/AECENAR

بسم الله الرحمن الرحيم

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ACETYL CHLORIDE PRODUCTION

OVERVIEW

•**Background**: White phosphorus is a crucial material in various chemical processes, including chemical vapor deposition and semiconductor applications. The need for high purity white phosphorus drives the development of efficient preparation methods.

•**Objective**: To present a method for converting high purity red phosphorus to high purity white phosphorus.

$$CH_3COOH + PCl_5 \rightarrow CH_3COCl + POCl_3 + HCl$$

MATERIALS AND METHODS

Materials:

- High purity red phosphorus (≥ 99% purity)
- Pyrex glass apparatus (bulbs, tubes)
- Heating furnace
- Nitrogen gas
- Whatman #50 filter paper
- Gas-tight syringe
- Oxygen torch

PROCEDURE

1. Preparation:

- The reaction should be set up in a fume hood due to the release of HCl gas.
- A dry reaction flask is equipped with a stirring mechanism.
- The flask is placed in an ice bath to control the exothermic reaction.

2. Addition of PCl_5:

3. Reaction:

- The reaction mixture is allowed to warm to room temperature and is stirred until the reaction is complete.

4. Purification:

Distillation: _ Acetyl chloride 51 °C
_Acetic acid 118 °C

CONDITIONS

1.Temperature:
The reaction is exothermic and should be initiated at low temperatures (0°C to 10°C) using an ice bath to control the heat and prevent excessive release of HCl gas.

2. Reaction Environment:
The reaction must be carried out in a water-free environment to avoid hydrolysis of acetyl chloride. It should be performed in a well-ventilated fume hood to safely manage the HCl gas produced.

3. Reagents Ratio:
A typical molar ratio of 1:1 for acetic acid to PCl_5 is used, but excess PCl_5 can be added to ensure complete conversion.

4. Stirring:
Continuous stirring is crucial for proper mixing, smooth reaction progress, and effective heat dissipation.

5.Time:

The reaction usually takes 30 minutes to 1 hour to complete, depending on the scale and specific conditions

Ahmad Jawhar @MEGBI/AECENAR
Ali Foul @MEGBI/AECENAR

(Poster from Sep 2024)

بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

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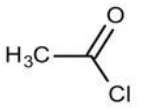
Association for Economical and Technological Cooperation in the Euro-Asian and North-African Region

Acetyl chloride production scale

Introduction: Acetyl chloride (CH_3COCl) is an acyl chloride used in various organic synthesis reactions, particularly for introducing acetyl groups. One common method of synthesis is by reacting acetic acid with phosphorus pentachloride (PCl_5), a strong chlorinating agent.

Alternate Name:
1.Acetic acid chloride
2.Ethanoyl Chloride
3.Acyl Chloride



Chemical structure

Objective: This poster explores the chemical synthesis process of acetyl chloride and the reaction conditions necessary for optimal production

Chemical reaction:

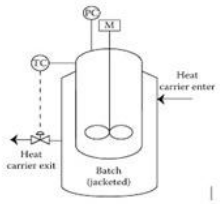
$$CH_3COOH+PCl_5 \rightarrow CH_3COCl+POCl_3+HCl$$

In this reaction, phosphorus pentachloride replaces the hydroxyl group (-OH) in acetic acid with a chlorine atom, forming acetyl chloride. The by-products are phosphorus oxychloride
($POCl_3$) and hydrogen chloride (HCl)

Parameter	condition	Explanation
Time	1to 2 hours	
Reaction type	Exothermic	Heat is released during the reaction, which needs to be controlled.
Temperature	20–40°C (room temperature to slightly elevated)	Optimal for the reaction, prevents decomposition of acetyl chloride.
Pressure	Optimal for the reaction, prevents decomposition of acetyl chloride.	No pressure requirements

Unit operation needed:

Reactor : Since this is a relatively simple, exothermic reaction, a batch reactor may be appropriate for small-scale synthesis, allowing for careful control of reactant addition



3_phase separator :
3-phase separator in acetyl chloride synthesis separates hydrogen chloride gas (HCl), acetyl chloride (light liquid), and phosphorus oxychloride (heavy liquid). HCl gas is vented to a scrubber, while acetyl chloride and phosphorus oxychloride are separated based on density. This ensures efficient separation and safe handling of corrosive by-products.

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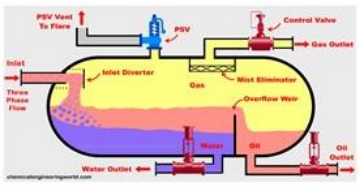
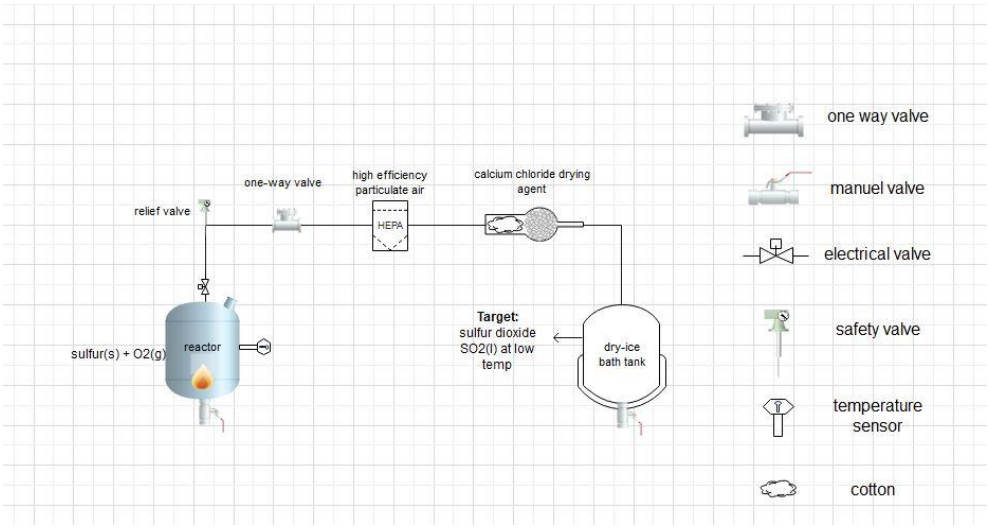


Rawan Abdelmajid @ AECENAR_Green chemistry /September 2024

Poster of Acetyl chloride production scale [pptx file]:	10-09-24Acetyl chloride production

19.6 Needed Production Sections for Aspirin Production Plant

<table>
<tr><th>Needed Place</th><th>Production Section</th><th colspan="2"></th></tr>
<tr><td rowspan="2">3mx2m</td><td rowspan="2">(0) Sulfur Dioxid Production</td><td colspan="2"> 
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 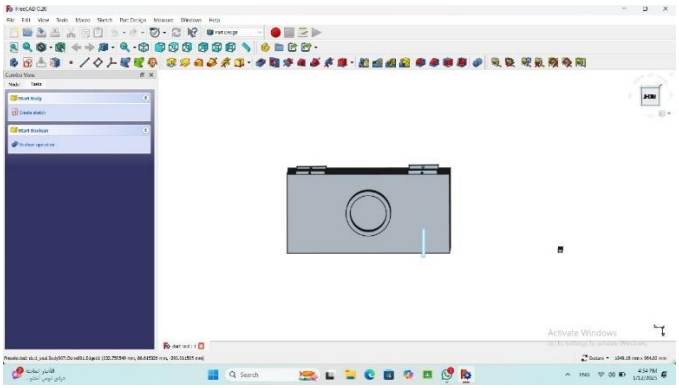 </td><td>https://drive.google.com/file/d/1D4bzIMto5m_PWutzEXQCJMI58VJmh7fx/view?usp=drive_link (Duct seal)</td></tr>
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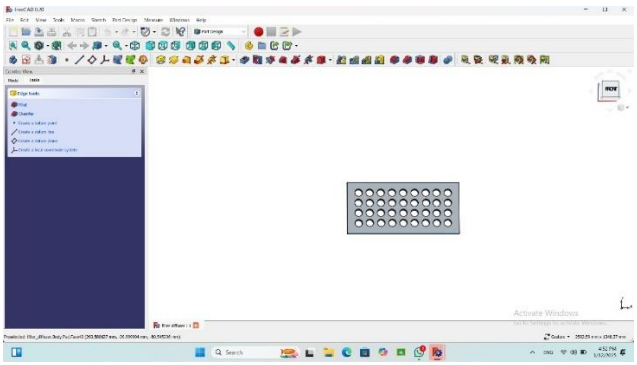
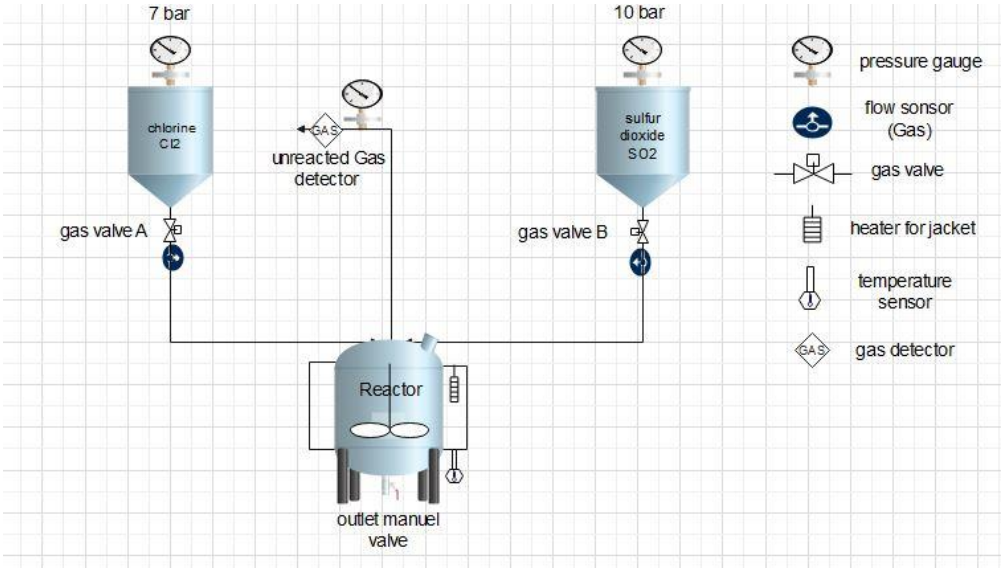
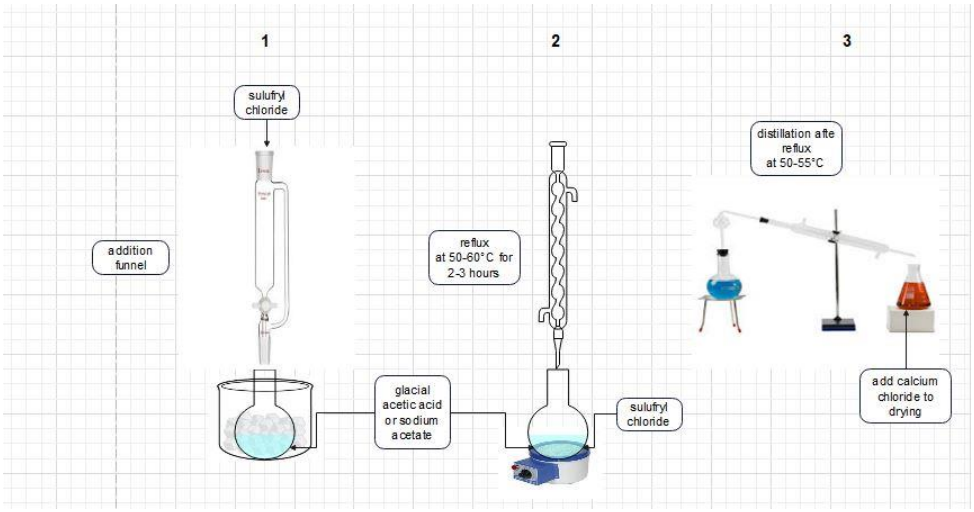
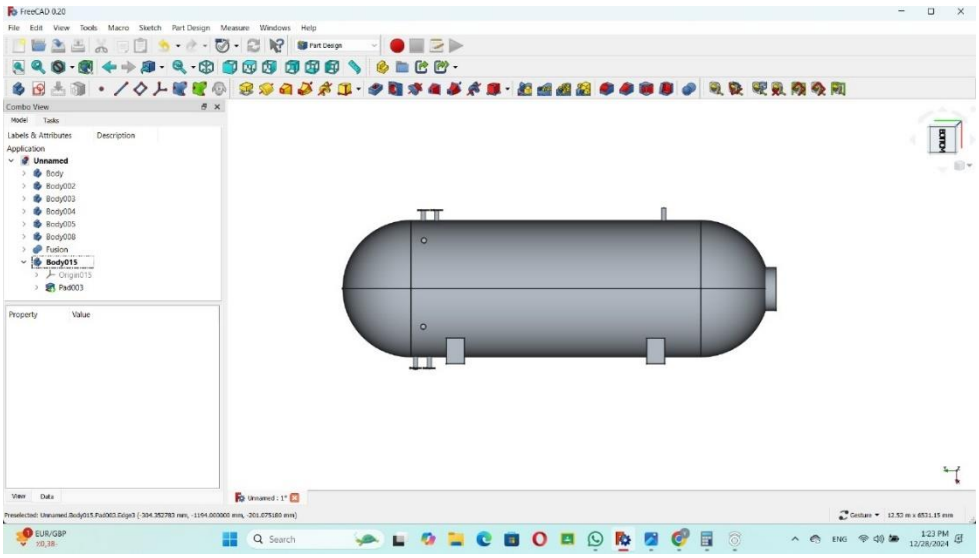
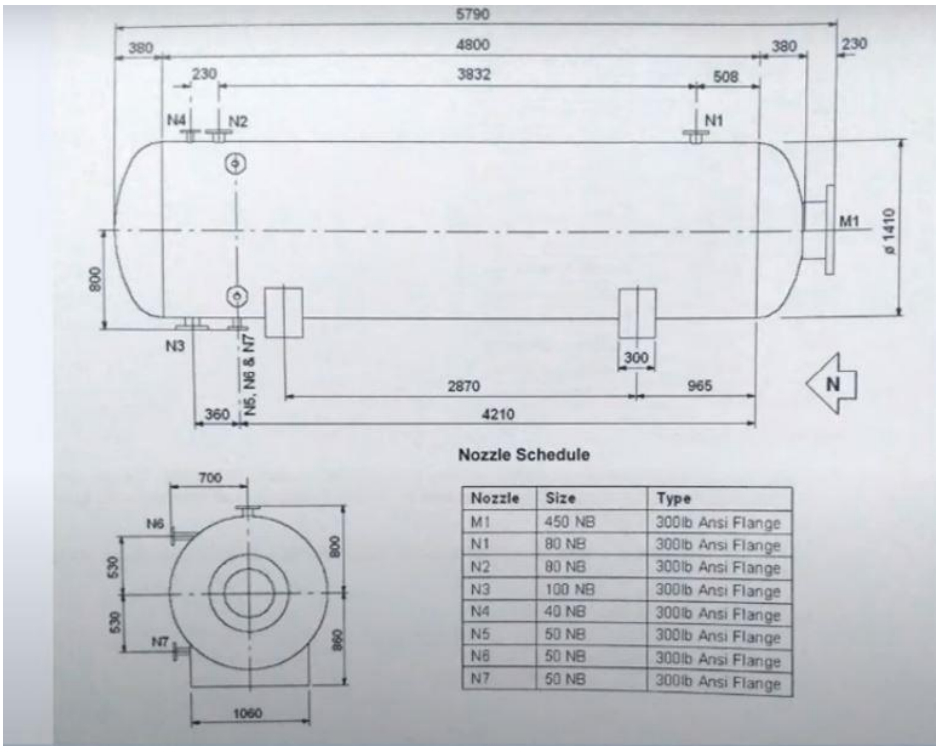
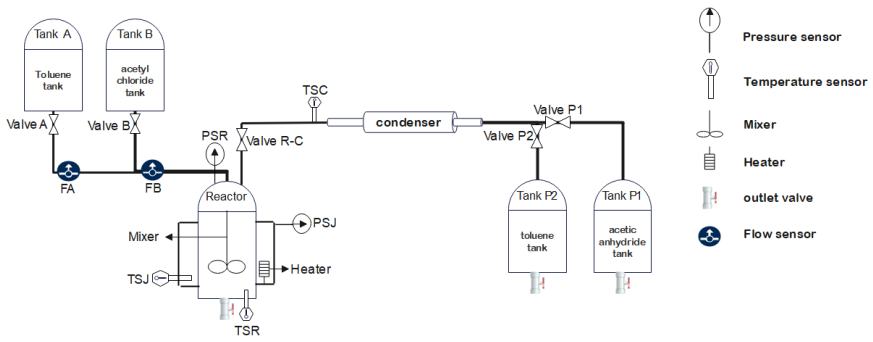
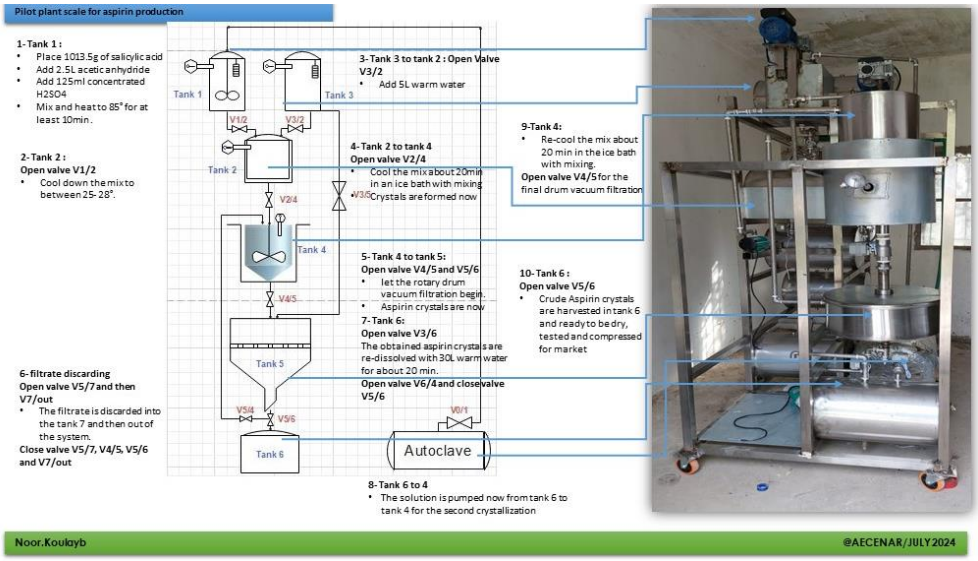
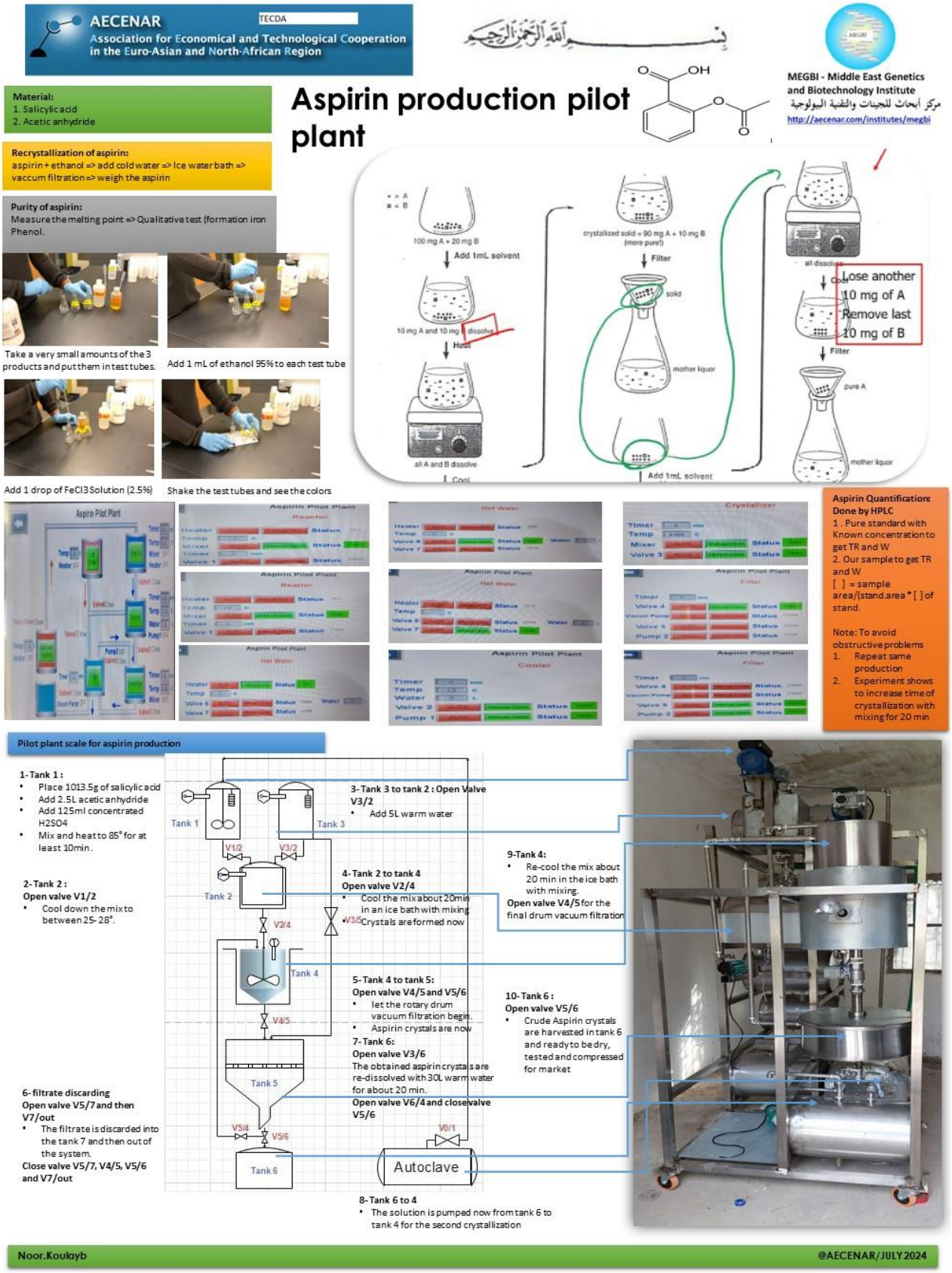
			https://drive.google.com/file/d/1JgdSB8Hpipp6wvUrTj0-zpD-pHu8E9gB/view?usp=drive_link (Filter diffuser)
3mx2m	(1) Sulfuryl chloride Production		
3mx2m	(2a) Acetyl Chloride Production		

		 Reflux drum: https://drive.google.com/file/d/19OzRXjUFxW4LpvQ9NoZQcnv-FAnyDO2U/view?usp=drive_link 
3mx2m	(3) Sodium Acetat Production	

3mx2m	(4) Acetic Anhydride Production	
3mx2m	(5) Aspirin Production	
3mx12m	***Total Plant***	

20 Project 1: Aspirin Production Project

20.1 Poster concerning the Aspirin production pilot plant



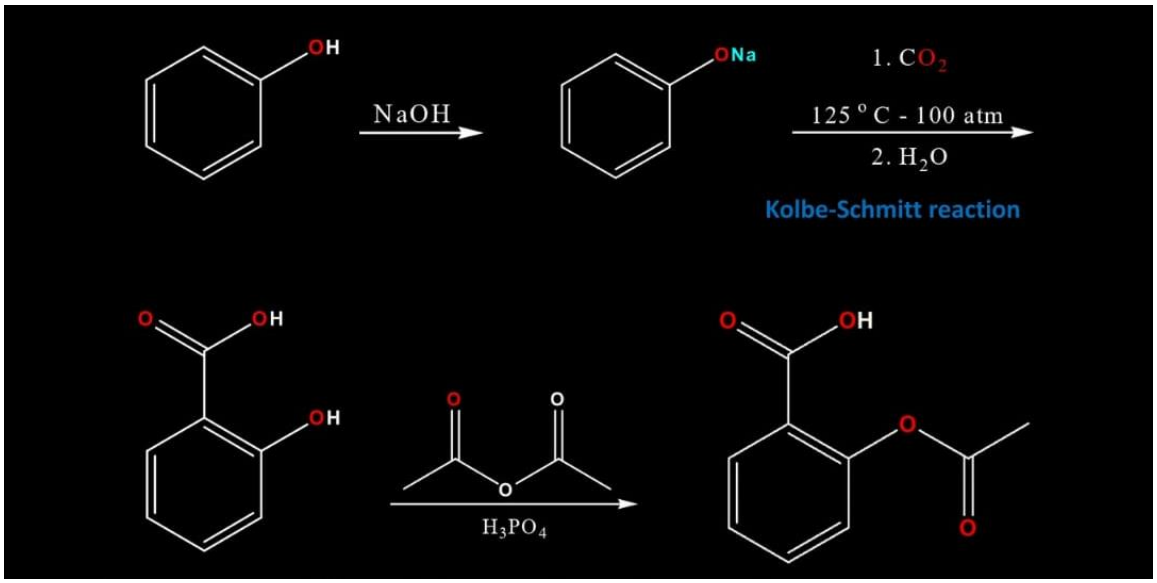
20.2 Aspirin production

20.2.1 Introduction

Aspirin, also known as acetylsalicylic acid (ASA), is a medication used to reduce pain, fever, or inflammation. Aspirin is used to treat specific inflammatory conditions including Kawasaki disease, pericarditis, and rheumatic fever. Aspirin given shortly after a heart attack decreases the risk of death.

Aspirin is also used long-term to help prevent further heart attacks, ischaemic strokes, and blood clots in people at high risk. It may also decrease the risk of certain types of cancer, particularly colorectal cancer.

20.2.2 Synthesis



20.2.3 Trademark

Bayer lost its trademark for Aspirin in the United States in actions taken between 1918 and 1921 because it had failed to use the name for its product correctly and had for years allowed the use of "Aspirin" by other manufacturers without defending the intellectual property rights. Today, aspirin is a generic trademark in many countries. Aspirin, with a capital "A", remains a registered trademark of Bayer in Germany, Canada, Mexico, and in over 80 other countries, for acetylsalicylic acid in all markets, but using different packaging and physical aspects for each.

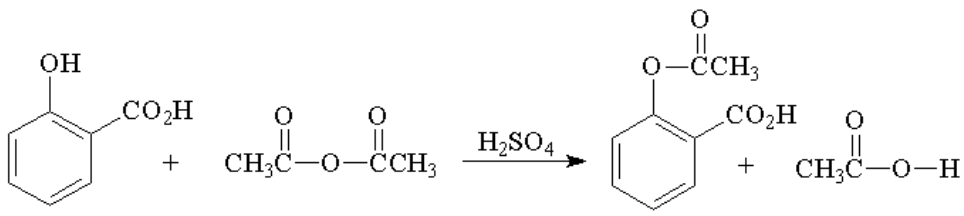
20.2.4 Adverse effects

In October 2020, the U.S. Food and Drug Administration (FDA) required the drug label to be updated for all nonsteroidal anti-inflammatory medications to describe the risk of kidney problems in unborn babies that result in low amniotic fluid. They recommend avoiding NSAIDs in pregnant women at 20 weeks or later in pregnancy. One exception to the recommendation is the use of low-dose 81 mg aspirin at any point in pregnancy under the direction of a health care professional.

20.2.5 References

- "Aspirin". Drugs.com. American Society of Health-System Pharmacists. 6 June 2016. Archived from the original on 25 April 2017.
- "FDA Warns that Using a Type of Pain and Fever Medication in Second Half of Pregnancy Could Lead to Complications". U.S. Food and Drug Administration (FDA)(Press release). 15 October 2020. Retrieved 15 October 2020. ‹See TfM› This article incorporates text from this source, which is in the public domain.
- "NSAIDs may cause rare kidney problems in unborn babies". U.S. Food and Drug Administration. 21 July 2017. Retrieved 15 October 2020. ‹See TfM› This article incorporates text from this source, which is in the public domain.
- Bayer Co. v. United Drug Co., 272 F. 505, p.512 (S.D.N.Y 1921).
- "Has aspirin become a generic trademark?". genericides.org. 25 March 2020. Retrieved 17 February 2021.
- Huth EJ, et al. (CBE Style Manual Committee) (1994). Scientific style and format: the CBE manual for authors, editors, and publishers. Cambridge University Press. p. 164. Bibcode:1994ssfc.book.....S. ISBN 978-0-521-47154-1. Archived from the original on 15 October 2015.
- "Aspirin: the versatile drug". CBC News. 28 May 2009. Archived from the original on 6 November 2016.
- Cheng TO (2007). "The history of aspirin". Texas Heart Institute Journal. 34 (3): 392–3. PMC 1995051. PMID 17948100.

Aspirin Synthesis (Acetylsalicylic acid)



20.2.6 Procedure

Procedure 1st part

1. Place 2.0 g (0.015 mole) of salicylic acid in a 125-mL Erlenmeyer flask.
2. Add 5 mL (0.05 mole) of acetic anhydride, followed by 5 drops of conc. H_2SO_4 (*use a dropper, H_2SO_4 is highly corrosive*) and swirl the flask gently until the salicylic acid dissolves.
3. Heat the flask gently on the steam bath for at least 10 minutes.
4. Allow the flask to cool to room temperature. If acetylsalicylic acid does not begin to crystallize out, scratch the walls of the flask with a glass rod. Cool the mixture slightly in an ice bath until crystallization is completed. The product will appear as a solid mass when crystallization is completed.
5. Add 50 mL of water and cool the mixture in an ice bath. Do not add the water until crystal formation is complete.
6. Vacuum filter the product using a Buchner funnel. You can use some of the filtrate to rinse the Erlenmeyer flask if necessary.

Rinse the crystals several times with small portions (5 mL) of cold water and air dry the crystals on a Buchner funnel by suction until the crystals appear to be free of solvent. Test this crude product for the presence of unreacted salicylic acid using the ferric chloride test. Record the weight of the crude solid which probably contains water.

Precedure 2nd part

1. Stir the crude solid with 25 mL of a saturated aqueous sodium bicarbonate solution in a 150 mL beaker until all signs of reaction have ceased (evolution of CO_2 ceases).
2. Filter the solution through a Buchner funnel to remove any insoluble impurities or polymers that may have been formed. Wash the beaker and the funnel with 5 to 10 mL of water.
3. Carefully pour the filtrate with stirring, a small amount at a time, into an ice cold HCl solution (*ca* 3.5 mL of conc. HCl in 10 mL of water) in a 150-mL beaker and cool the mixture in an ice bath. Make sure that the resulting solution is acidic (blue litmus paper) and that the aspirin has completely precipitated out.
4. Filter the solid by suction and wash the crystals 3X with 5 mL of *cold* water each. Remove all the liquid from the crystals by pressing with a clean stopper or cork. Air dry the crystals and transfer them to a watch glass to dry. Test a small amount of the product for the presence of unreacted salicylic acid using the ferric chloride solution.
5. When the product is completely dry, weigh the product, determine its melting point (lit mp 135-136 °C) and calculate the percentage yield.

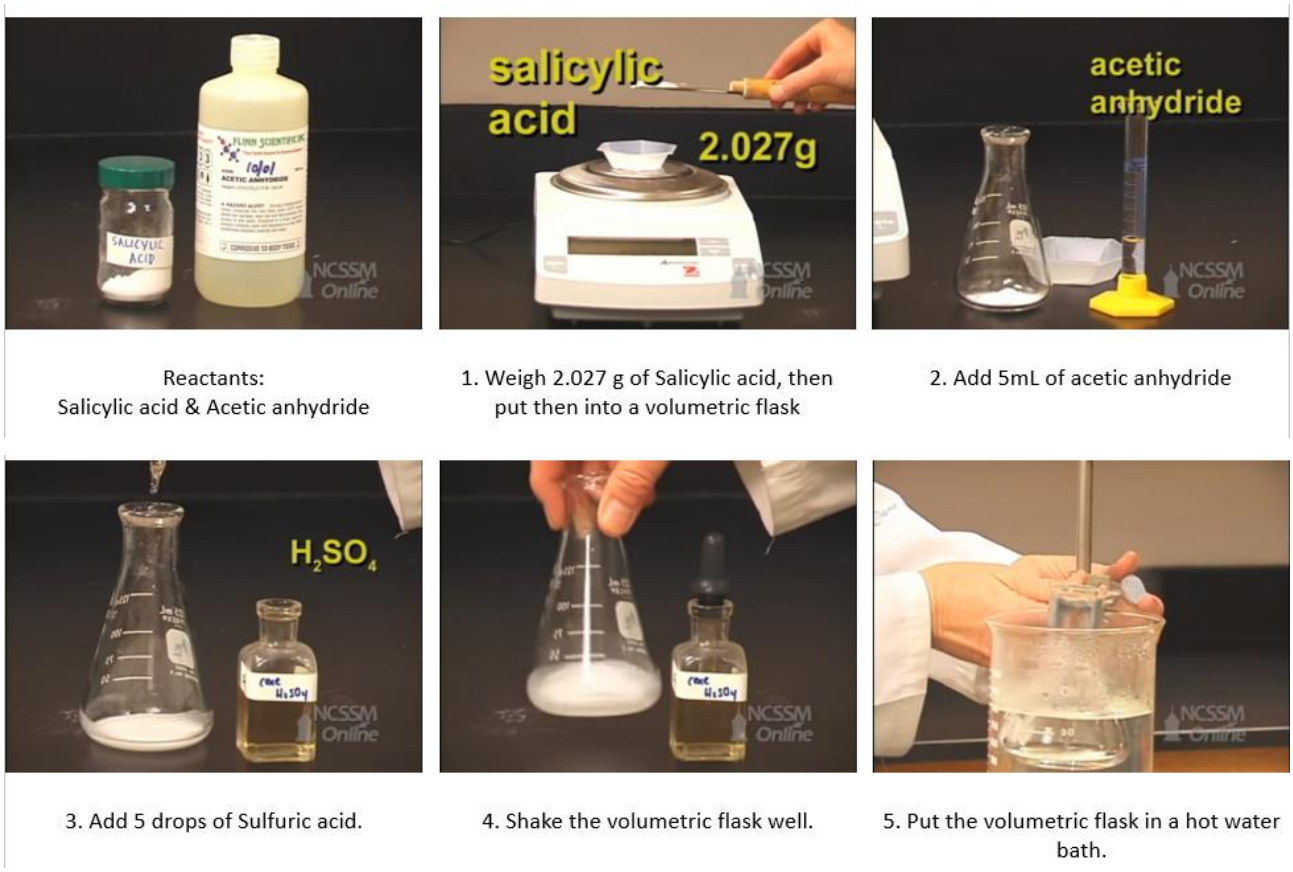
6. Dissolve the final product in a minimum amount (no more than 2-3 mL) of *hot* ethyl acetate in a 25 mL Erlenmeyer flask. Make sure that the product is completely dissolved while gently and continuously heating on a steam bath.
7. Cool the solution to room temperature and then in a ice-bath. Collect the product by vacuum filtration and rinse out of the flask with a few milliliters of cold petroleum ether.

When the product is completely dry, weigh its weight, determine its melting point (lit mp 135 °C) and calculate the percentage yield of this recrystallized product. Calculate the % recovery of recrystallized material from crude material. Submit the crystalline sample in a small vial with proper labeling to your instructor.

Aspirin production presentation [PPtx file]:

Aspirin.pptx

20.3 Aspirin Procedure in LAB Scale



Reactants:
Salicylic acid & Acetic anhydride

1. Weigh 2.027 g of Salicylic acid, then put then into a volumetric flask

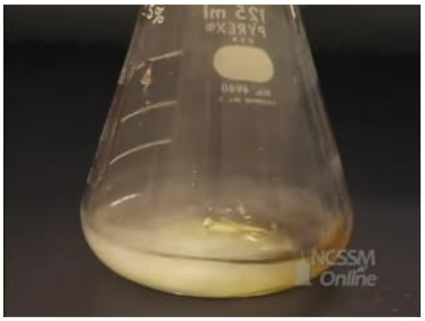
2. Add 5mL of acetic anhydride

3. Add 5 drops of Sulfuric acid.

4. Shake the volumetric flask well.

5. Put the volumetric flask in a hot water bath.

6. After 10 minutes, let the volumetric flask cool down



7. Add 10 mL of warm water.

8. Put the volumetric flask in ice, so aspirin can start precipitating.

9. 10 minutes later, the aspirin should be precipitated.

10. Now, we filtrate the aspirin by a vacuum filtration.

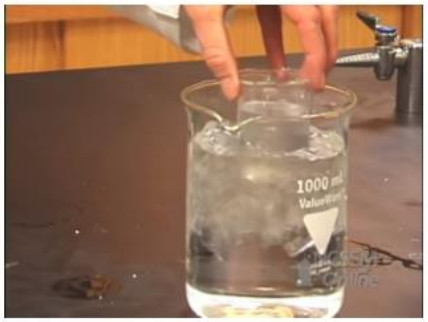
11. Add small amount of cold water on the filter paper.

12. Add the solution.

13. Pour the crystals into a beaker.

14. Dissolve them again by adding water.

15. Add 60 mL of warm water.

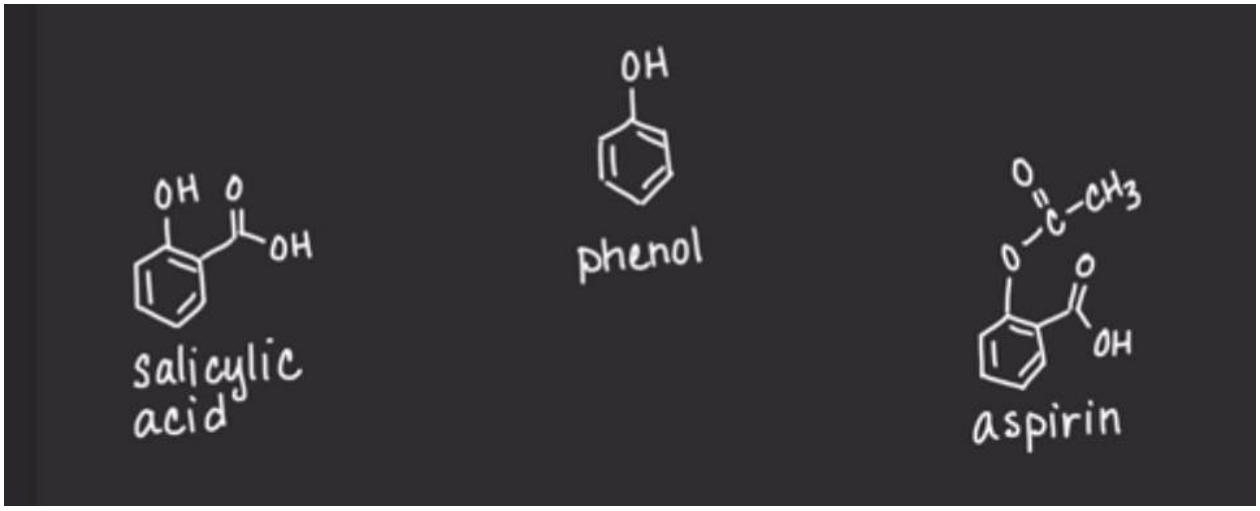


16. Put the beaker in ice to recrystallize.

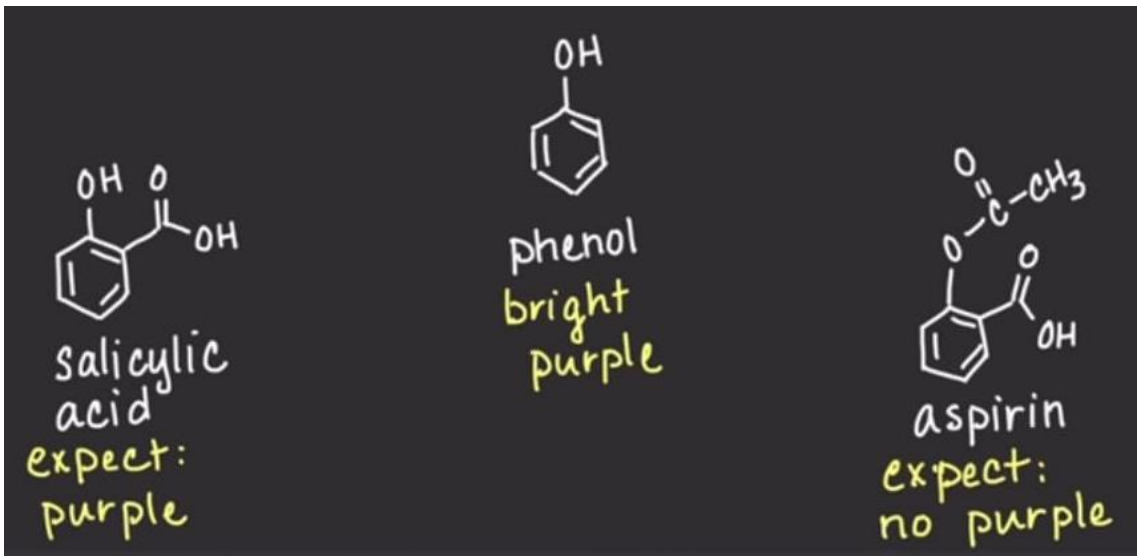
17. Last step, filter the product again.

Note: **In step 11 and 17 we used standard filtration, not vacuum filtration**

Synthesis of Aspirin – FeCl3 Test

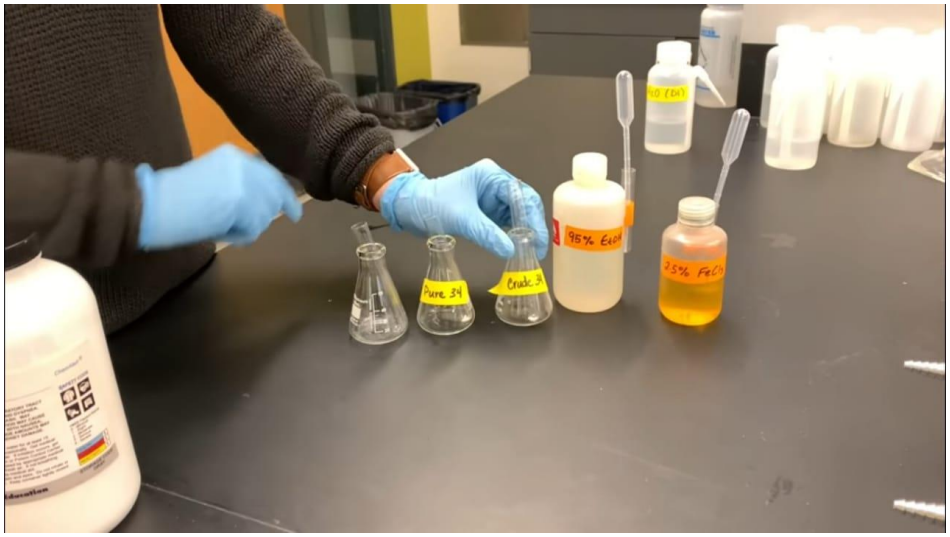


FeCl3 test, which is a qualitative test

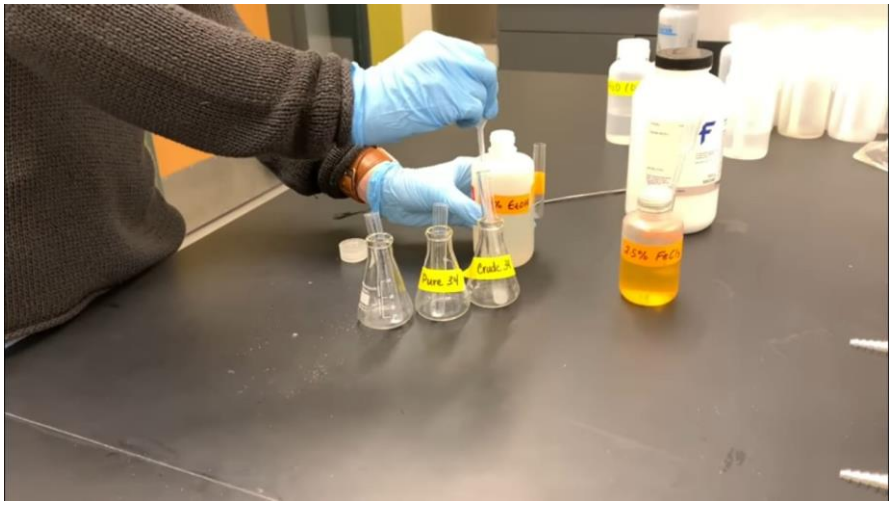


Expected colors! Since FeCl3 reacts with the OH group

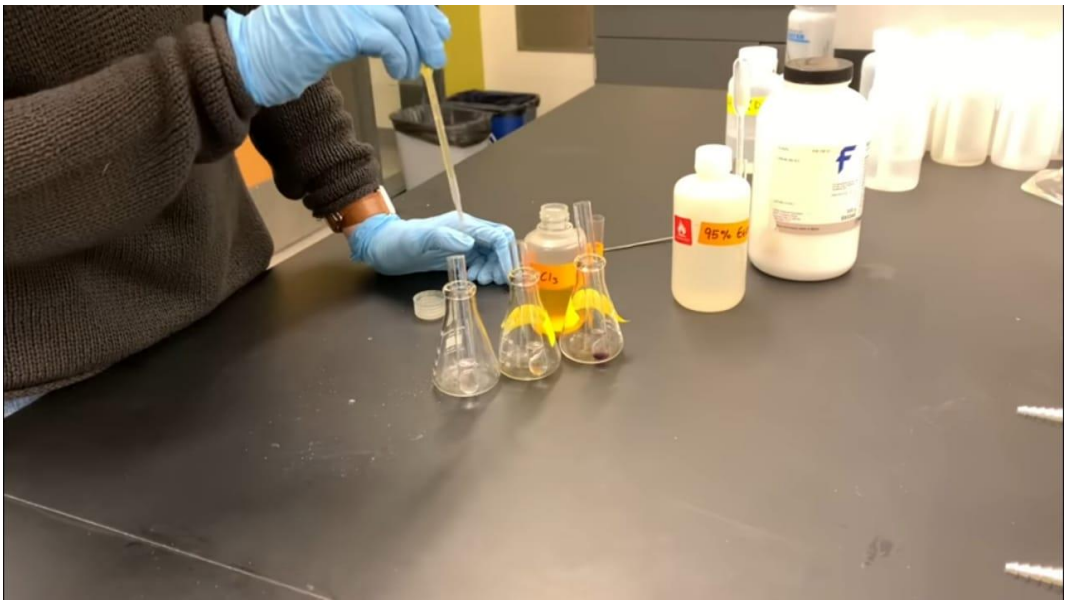
We can do the test for the crude product, pure product, and the salicylic acid



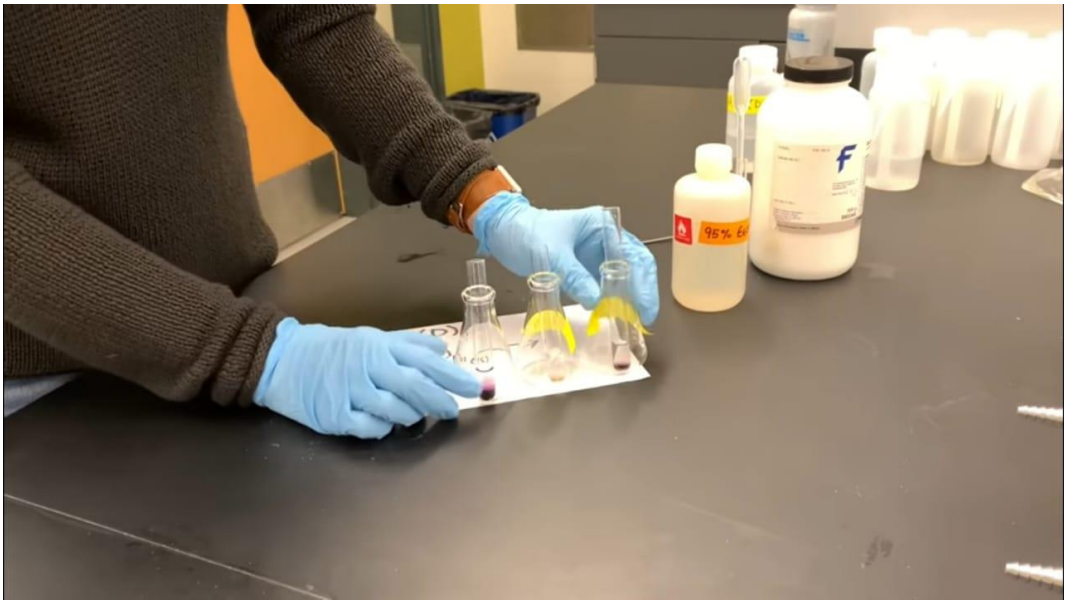
1. Take a very small amount of the 3 products and put them in test tubes.



2. Add 1 mL of ethanol 95% to each test tube



3. Add 1 drop of FeCl3 Solution (2.5%)



4. Shake the test tubes and see the colors.

21 Project 2: Acetic Anhydride Production Project

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ACETIC ANHYDRIDE PRODUCTION

Acetic Anhydride: A Versatile Chemical Intermediate Acetic anhydride is a key organic compound with diverse applications in various industries. This poster explores its synthesis, properties, and key applications, highlighting its importance in modern chemical manufacturing.

$$CH_3COCl(l) + CH_3COONa(s) \rightarrow (CH_3CO)_2O(l) + NaCl(s)$$


Add sodium acetate in the round bottom flask

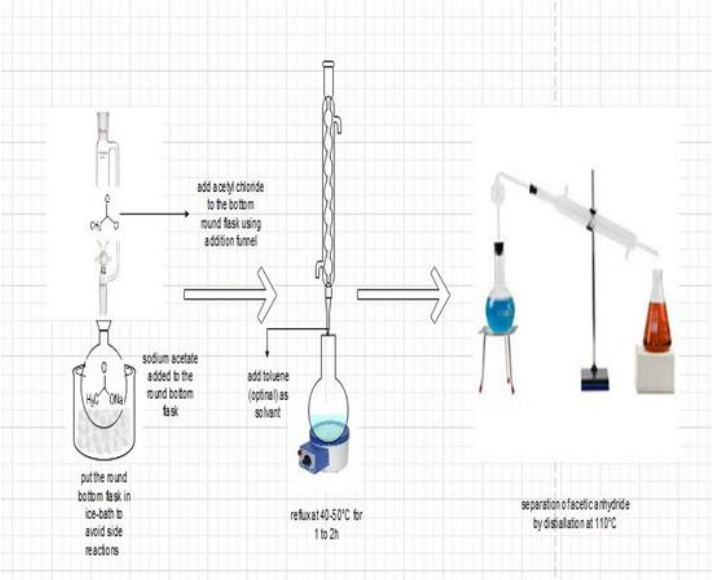

Add acetyl chloride using addition funnel


Put gas safety outlet


Cooling the reaction in the round bottom flask by an ice bath


Reflux at 40-50 °C for 1-2h


Distillation of acetic anhydride at 110 °C



MATERIALS AND METHODS

Apparatus Setup:

•Set up a dry, well-ventilated reaction apparatus equipped with:

- A round-bottom flask with a magnetic stirrer
- A reflux condenser
- A dropping funnel
- A thermometer

PREPARATION OF REAGENTS

•**Dry Sodium Acetate:** Anhydrous sodium acetate is crucial. If not available, dry sodium acetate trihydrate in an oven at 120-140°C for several hours. Store in a desiccator.

•**Dry Solvent:** If using a solvent (e.g., anhydrous diethyl ether or dichloromethane), ensure it is properly dried over a suitable drying agent (e.g., calcium chloride) and distilled.

Reaction:

•**Charge:** Place the calculated amount of dry sodium acetate in the round-bottom flask.

•**Solvent Addition:** If using a solvent, add the appropriate amount.

•**Cool Down:** Cool the flask in an ice bath.

•**Acetyl Chloride Addition:** Slowly add acetyl chloride dropwise from the dropping funnel while maintaining the temperature below 10°C. Stir continuously.

•**Warm Up:** After complete addition, slowly warm the reaction mixture to room temperature.

•**Reflux:** Gradually increase the temperature to 40-50°C and reflux for 1-2 hours.

•**Distillation:** After the reaction is completed, turn ON the water circulation in the condenser to start the distillation by turning ON the heating to 110°C to evaporate the acetone
increase the temperature to 140°C to evaporate the Acetic Anhydride

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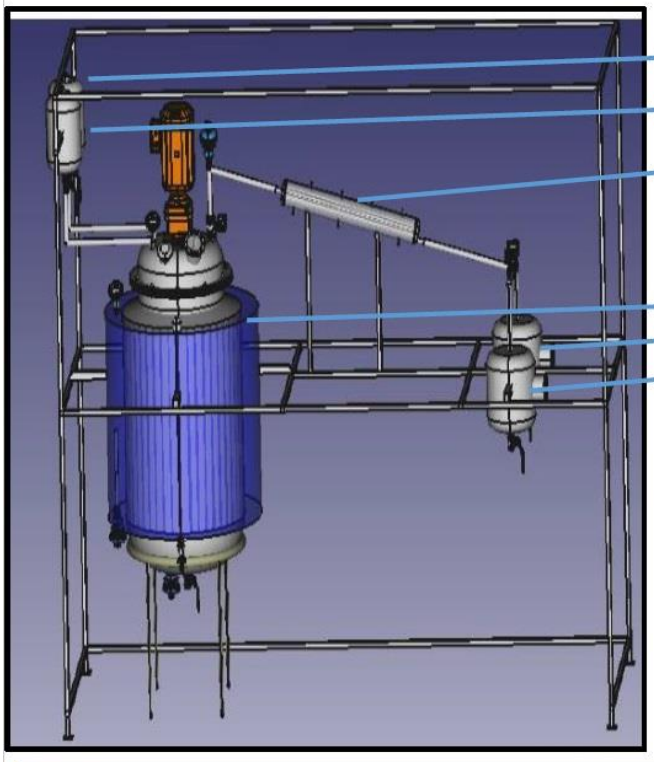
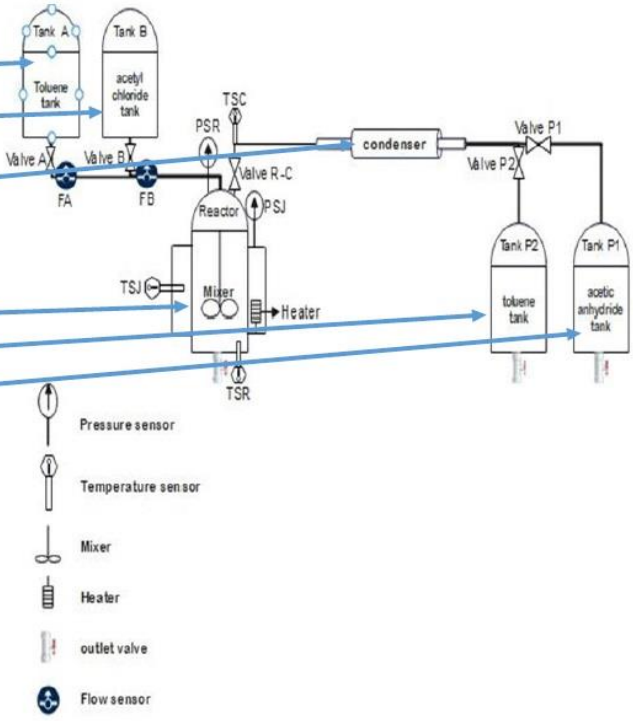
MEGBI - Middle East Genetics and Biotechnology Institute
مركز أبحاث للجينات والتقنية البيولوجية
http://aecenar.com/institutes/megbi

Acetic Anhydride

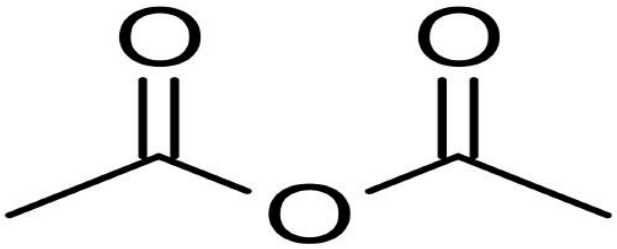




Reaction:
Sodium acetate(NaCH3COO) + acetic acid (CH3COOH) → H2O+CO2+NaCH3COO
2.Sodium acetate(NaCH3COO) + acetyl Chloride(CH3COCl) => acetic anhydride((CH3CO)2O) + NaCl

Material:
- Vinegar
- Sodium bicarbonate
- Acetyl chloride (C2H3ClO)

Safety:
1.Toluene: Sweet aroma,
2. Acetyl chloride: Corrosive, flammable
3.Acetic anhydride: Highly corrosive, inhalation damages lungs,



Procedure:
1. Mixer activation
2. Reagent addition for reaction 1"vinger + Na + C2H3ClO"
3. Heat to 120°C
4. Cool Down to 50-80°C
5. Add 5L of toluene as "solvent"
6. Distillation
7. Test purity and yield of acetic anhydride.

Requirements:
1. Physical: Pipes, tanks, and autoclave must withstand high temperatures (T = 300°C) and pressures (P = 260 bar).
2. Chemical: - Tanks, autoclaves, pipes, valves shall be withstand corrosion with acetic anhydride, toluene , acetic acetate and acetyl chloride
3. Mechanical:
system: stain less steel 316, close
Valve: close compound
Sensor: road data from system

Noor.Koulayb @AECENAR/JULY 2024

For aspirin we need as raw material acetic anhydride. So the goal of is to produce Acetic Anhydride [$(CH_3CO)_2O$] according to the following reaction:

Sodium Acetate + Acetyl Chloride → Acetic Anhydride + Sodium Chloride

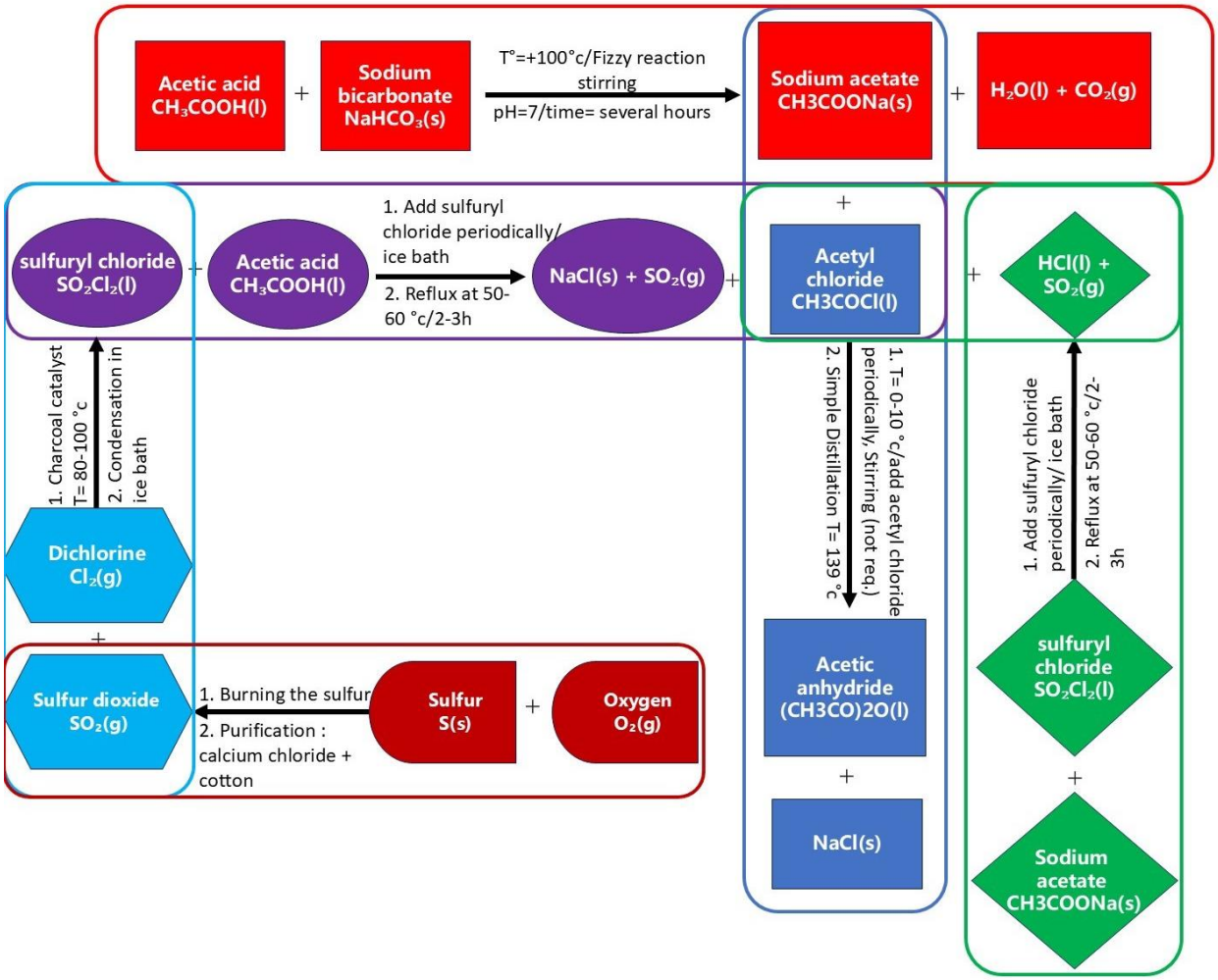
$CH_3COONa_{(s)} + CH_3COCl_{(l)} \rightarrow (CH_3CO)_2O_{(l)} + NaCl_{(s)}$

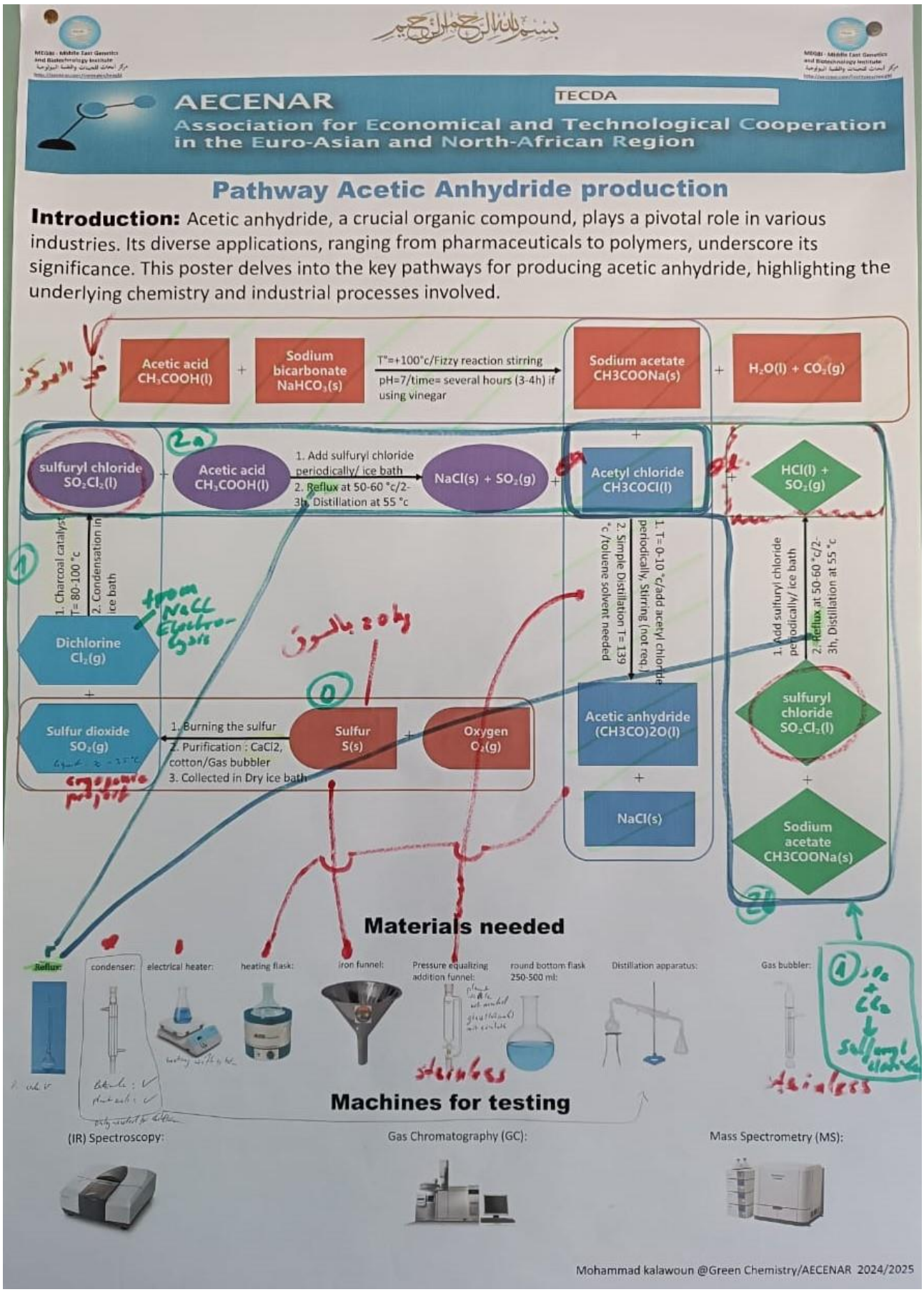
This reaction occurs under the following conditions:

1) T = 0-10°C, Add Acetyl Chloride periodically, Stirring (not req.)
2) Simple distillation at T = 139°C / Toluene solvent needed

To achieve this reaction, Sodium Acetate and Acetyl Chloride must be obtained.

And therefore we need to produce Acetyl Chloride.




بسم الله الرحمن الرحيم
AECENAR
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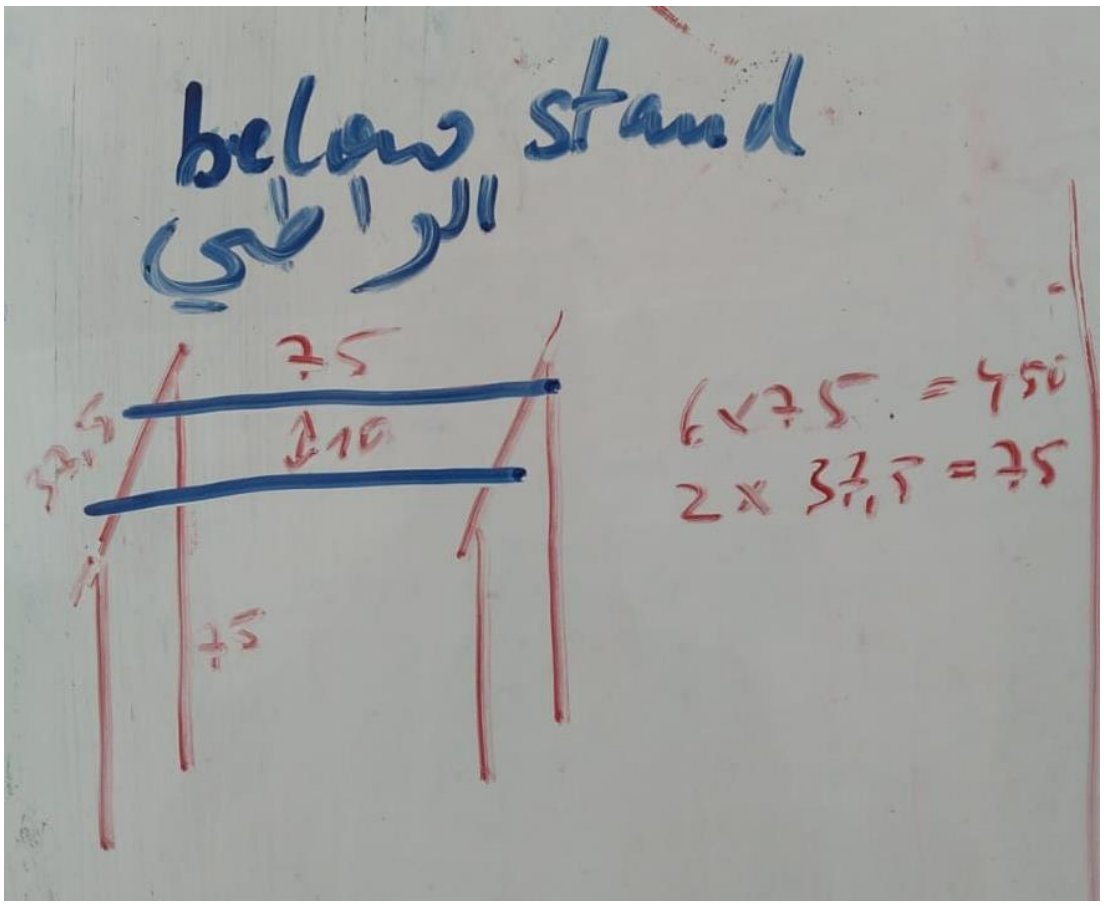
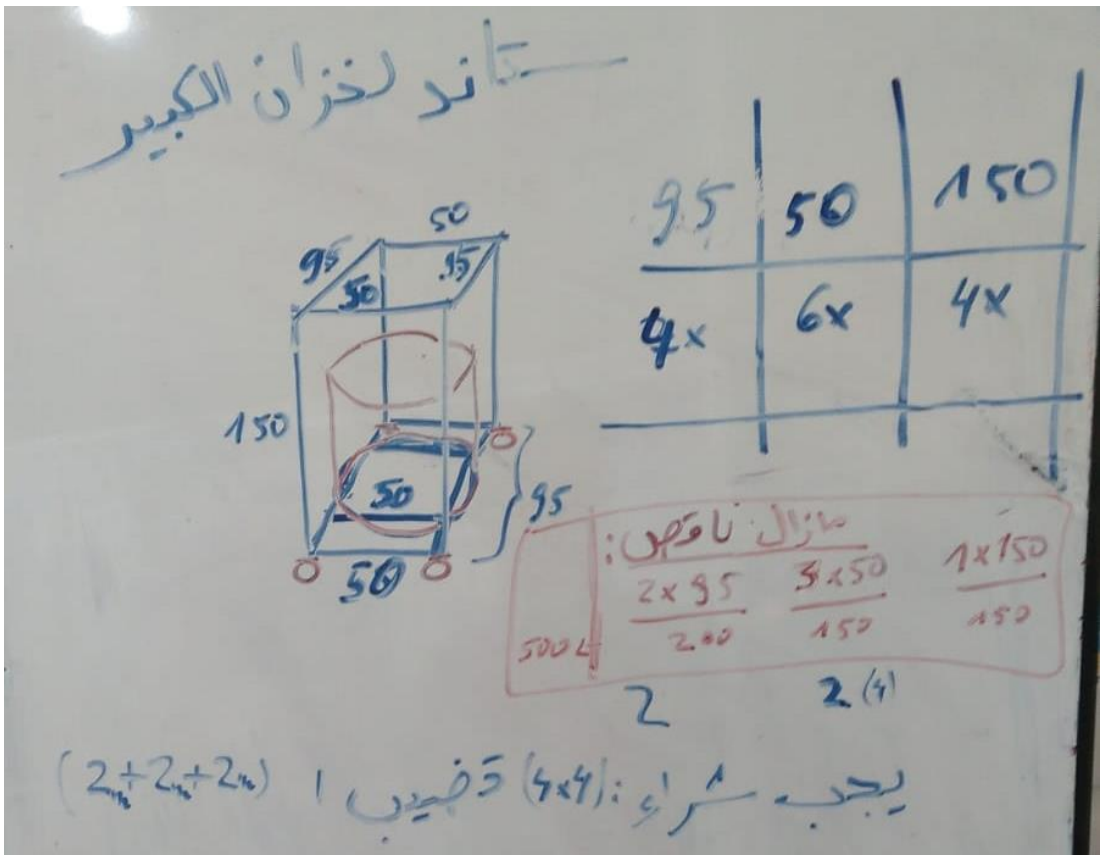
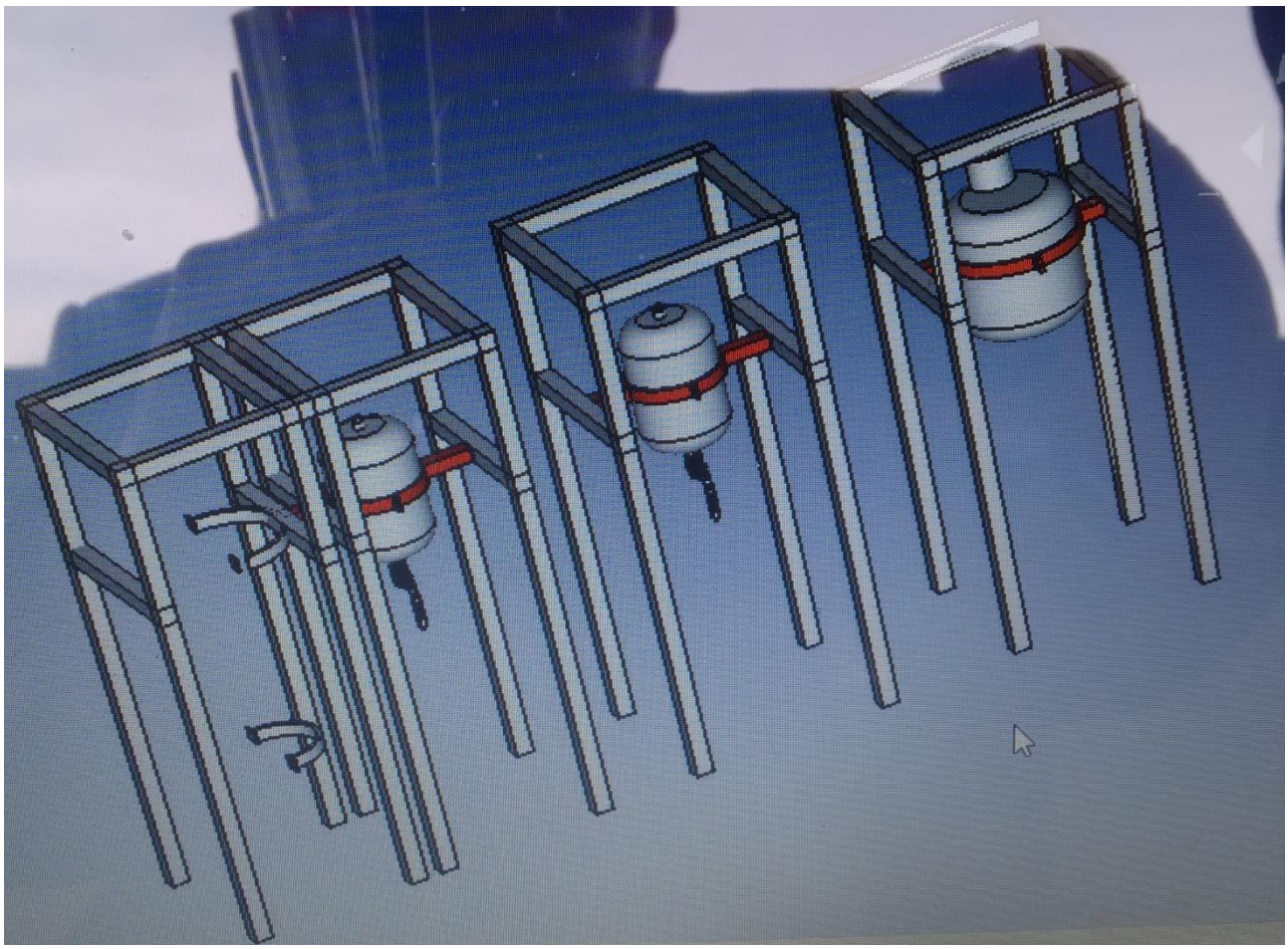
Pathway Acetic Anhydride production
Introduction: Acetic anhydride, a crucial organic compound, plays a pivotal role in various industries. Its diverse applications, ranging from pharmaceuticals to polymers, underscore its significance. This poster delves into the key pathways for producing acetic anhydride, highlighting the underlying chemistry and industrial processes involved.
Acetic acid CH3COOH(l)
Sodium bicarbonate NaHCO3(s)
T°=+100°c/Fizzy reaction stirring
pH=7/time= several hours (3-4h) if using vinegar
Sodium acetate CH3COONa(s)
H2O(l) + CO2(g)
sulfuryl chloride SO2Cl2(l)
Acetic acid CH3COOH(l)
1. Add sulfuryl chloride periodically/ ice bath
2. Reflux at 50-60 °c/2-3h, Distillation at 55 °c
NaCl(s) + SO2(g)
Acetyl chloride CH3COCl(l)
HCl(l) + SO2(g)
1. Charcoal catalyst T= 80-100 °c
2. Condensation in ice bath
Dichlorine Cl2(g)
from NaCl Electro-Gas
1. T= 0-10 °c/add acetyl chloride periodically, Stirring (not req.)
2. Simple Distillation T= 139 °c /toluene solvent needed
1. Add sulfuryl chloride periodically/ ice bath
2. Reflux at 50-60 °c/2-3h, Distillation at 55 °c
Sulfur dioxide SO2(g)
1. Burning the sulfur
2. Purification : CaCl2, cotton/Gas bubbler
3. Collected in Dry ice bath
Sulfur S(s)
Oxygen O2(g)
Acetic anhydride (CH3CO)2O(l)
NaCl(s)
sulfuryl chloride SO2Cl2(l)
Sodium acetate CH3COONa(s)
Materials needed
Reflux:
condenser:
electrical heater:
heating flask:
iron funnel:
Pressure equalizing addition funnel:
round bottom flask 250-500 ml:
Distillation apparatus:
Gas bubbler:
Machines for testing
(IR) Spectroscopy:
Gas Chromatography (GC):
Mass Spectrometry (MS):
Mohammad kalawoun @Green Chemistry/AECENAR 2024/2025

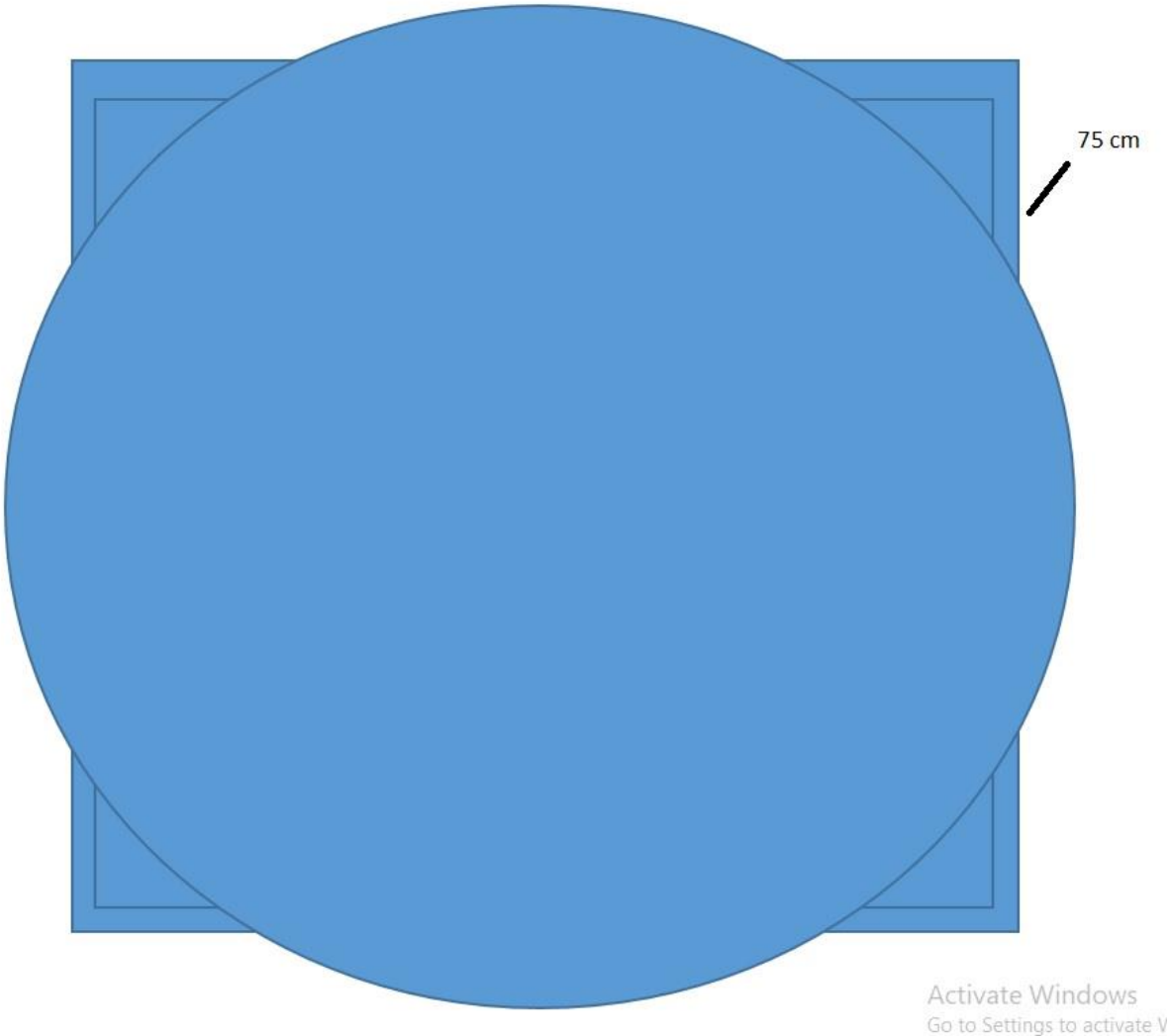
21.1 Plant Scale Devices

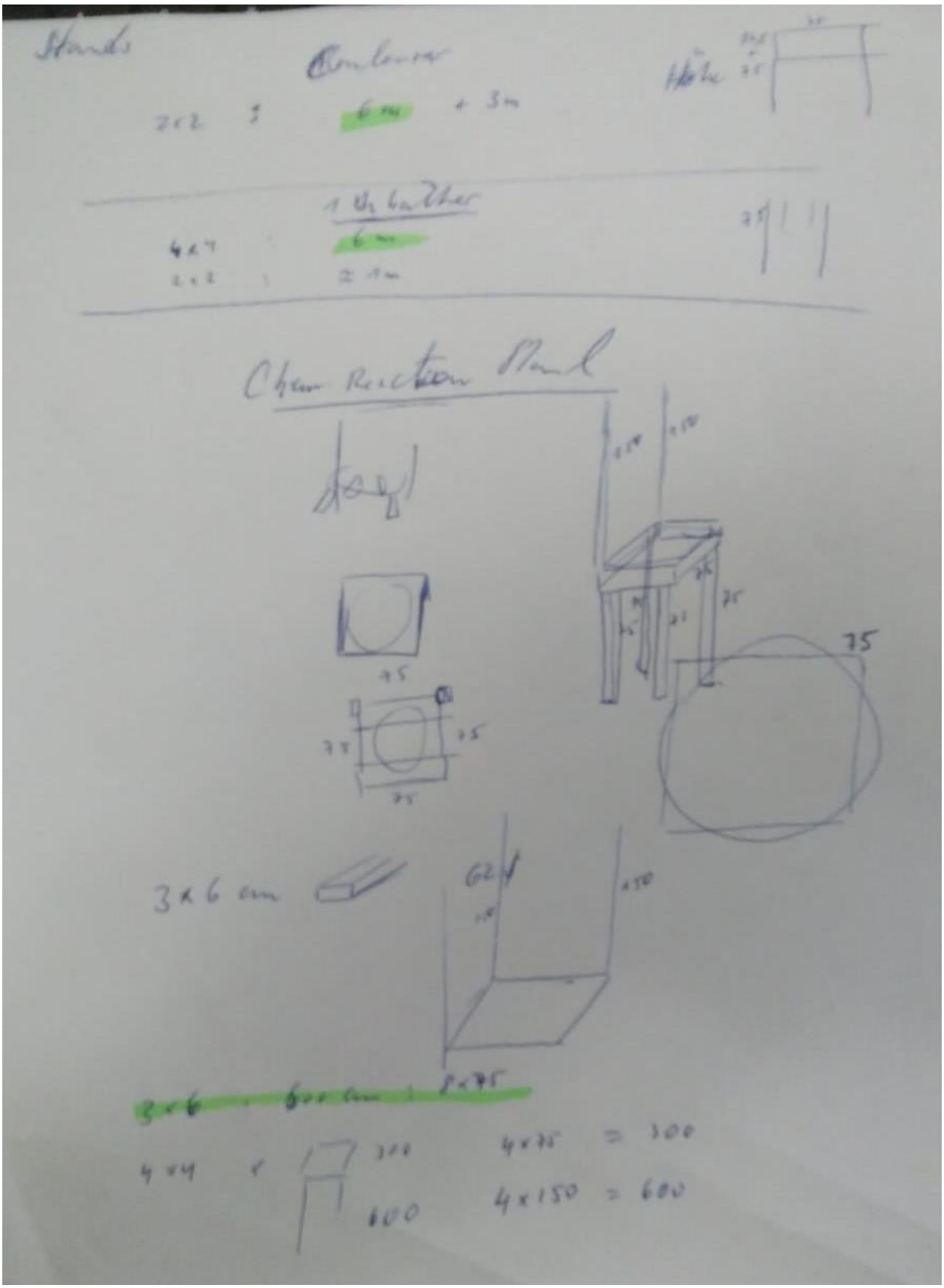

below stand
الراطي
75
37,5
110
75
6x75 = 450
2x37,5 = 75


ستاند لخزان الكبير
95
50
150
4x
6x
4x
50
95
150
50
95
50
نازل ناقص:
2x95
200
3x50
150
1x150
150
500 L
2
2 (4)
يجب شراء: (4x4) قطع 1
(2+2+2)




LASER FILM
LASER FILM
LASER FILM
LASER FILM





21.1.1 Needed Materials for Stands

1. Chemical React. Vessel:

3cm x 6cm (62$ per 6m): 8x75

Chemical React. Vessel	3cm x 6cm (62$ per 6m): 8x75	600 cm	62$
	4cm x 4cm (farigh)/ edges (zawiya) 4x4	4x150cm = 600cm	42$
	4 cm x 4cm (farigh)	4x75 cm = 300cm	21$

21.2 Production of acetic anhydride (chemical reactions and calculations)

21.2.1 Reactions

1. Sodium acetate + Acetic Acid → water + Carbone Dioxide + Sodium acetate

- $NaHCO_3(s) + CH_3COOH(l) \rightarrow CO_2(g) + H_2O(l) + CH_3COONa(s)$

2. Sodium acetate + Acetyl chloride → Acetic anhydride + Sodium chloride

- $CH_3COONa(s) + CH_3COCl(l) \rightarrow (CH_3CO)_2(l) + NaCl(s)$

21.2.2 Calculations

Acetic anhydride needed for 1 trial in aspirin process production = 5 Liters.

Theory calculation to know the amount of the following reagents needed for acetic anhydride production: (Acetic Acid "vinegar", Sodium acetate, Sodium Bicarbonate and Acetyl Chloride).

Acetyl chloride

Acetic anhydride
- $C_4H_6O_3$
- ρ = 1.08 g/ml
- M = 102.089 g/mol

$\rho = m/V \Rightarrow m = \rho.V \Rightarrow 1.08 \times 5000 = 5400$ g.

$n = m/M \Rightarrow 5400/102.089 = 52.896$ moles.

Calculate the volume of Acetyl Chloride:

$CH_3COONa(s) + CH_3COCl(l) \rightarrow (CH_3CO)_2(l) + NaCl(s)$ according to the reaction the ratio between Acetyl Chloride and Acetic Anhydride is (1:1), that's mean they have the same molar number.

- CH_3COCl

Acetyl Chloride ρ = 1.1 g/ml
M = 78.49 g/mol

ρ = m/V & m = n.M ⇒ V = n.M/ρ ⇒ 52.896 x 78.49 / 1.1 = 3.77 L.

Calculate the mass of Sodium Acetate:

In the same reaction mentioned above, Sodium acetate should be used it in excess to ensure the reaction is completed.

Sodium Acetate { CH_3COONa, ρ = 1.53 g/ml, M = 82.0343 g/mol

Here, the ratio of the excess should be a little higher; we choose according to the reference, the following ratio (1.1:1)

n = 52.895 x 1.1 = 58.1845 moles

n = m/M ⇒ m = n.M ⇒ 58.1845 x 82.0343 = 4.77 kg.

Calculate the mass of Sodium Bicarbonate:

$NaHCO_3(s) + CH_3COOH(l) \rightarrow CO_2(g) + H_2O(l) + CH_3COONa(s)$ according to the reaction the ratio between sodium bicarbonate and sodium acetate is (1:1), that's mean they have the same molar number.

Sodium Bicarbonate { $NaHCO_3$, ρ = 2.2 g/ml, M = 84.007 g/mol

m = n.M ⇒ 58.145 x 84.007 = 4.88 kg.

Calculate the volume of acetic acid 5% (vinegar):

In the same reaction mentioned above, Sodium acetate should be used it in excess to ensure the reaction is completed

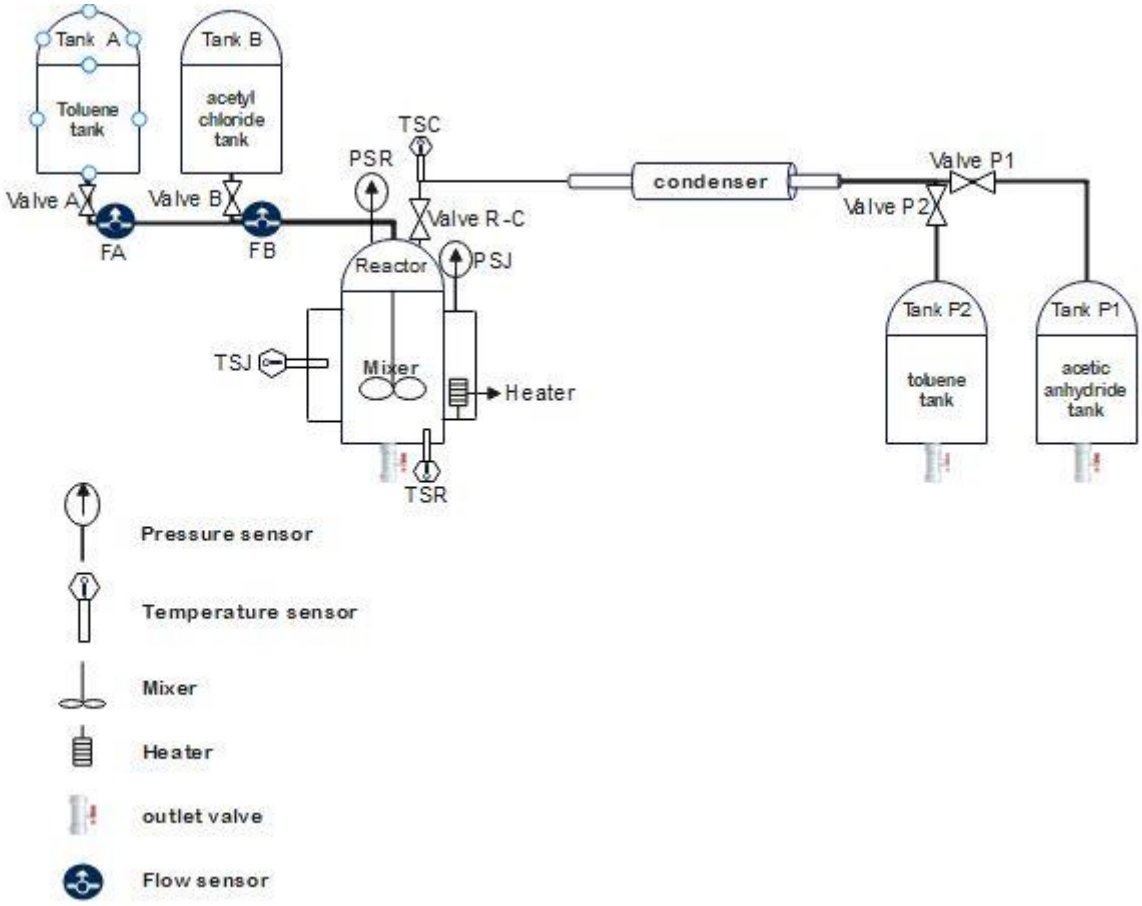
Acetic Acid { CH_3COOH, ρ = 1.0005 g/ml, C = 0.86 mol/l, M = 60.05 g/mol

Here, the ratio of the excess should be a little higher; we choose according to the reference, the following ratio (1.1:1)

The number of moles needed n x 1.1 = 58.1845 x 1.1 = 64.00295 moles.

C = n/V⇒ V= n/C = 64.00295/0.86 = 74.4 L.

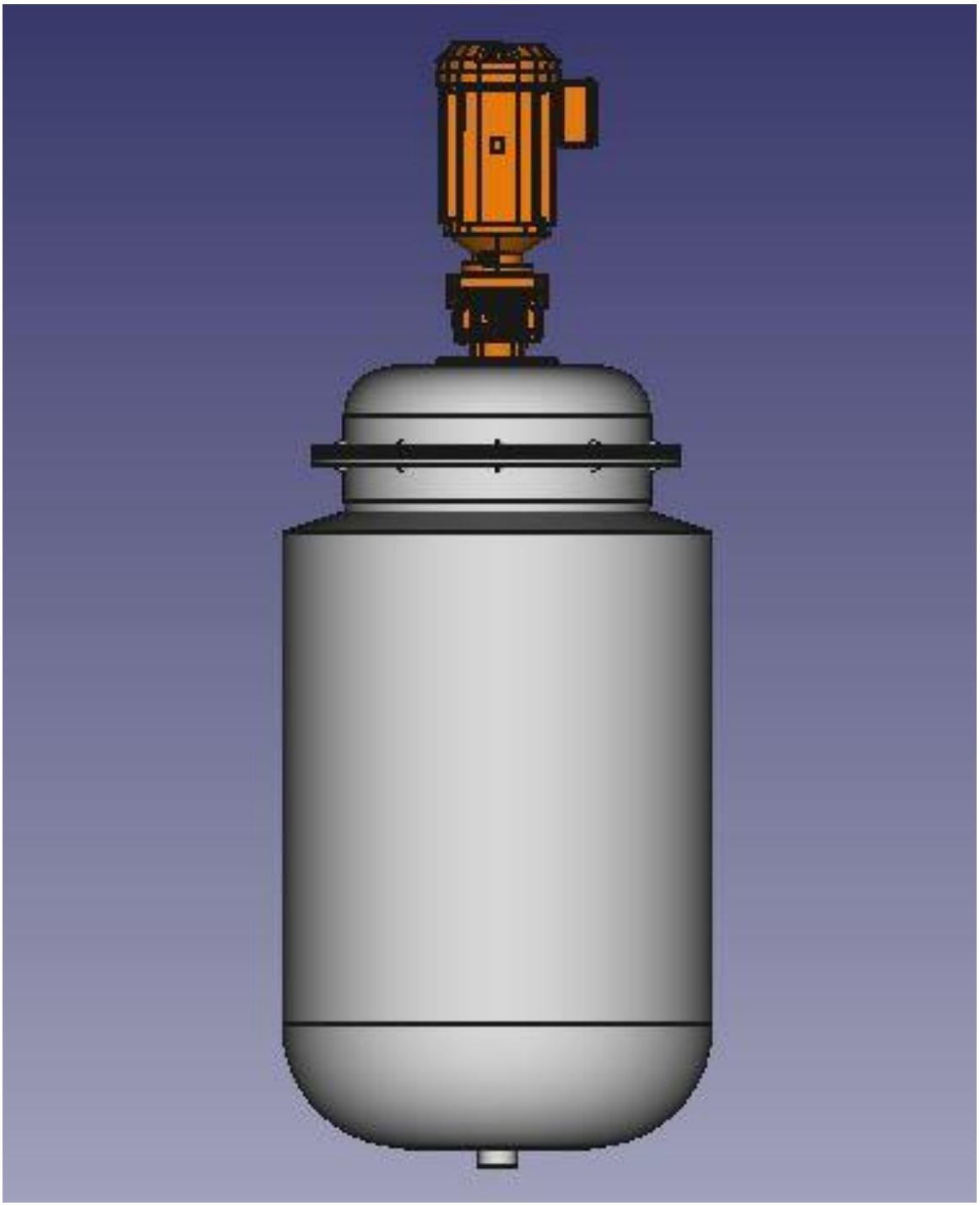
21.3 System design/system concept (Acetic Anhydride Pilot Plant productoin)



Approximate View for acetic anhydride pilot plant [Edraw file]:

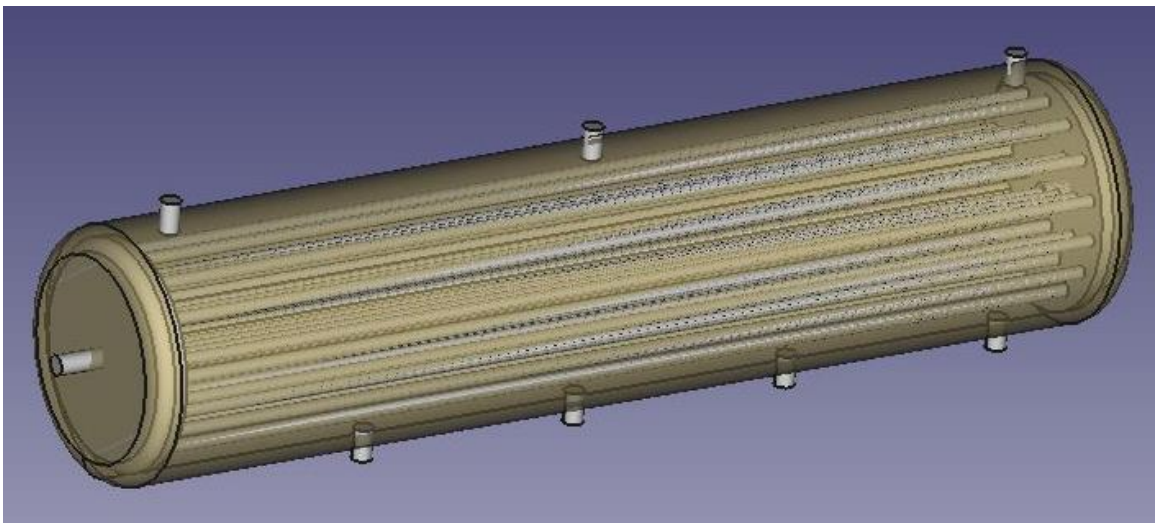
Edraw file acetic anhydride MAP for s

21.3.1 Mechanical design (Acetic Anhydride Pilot Plant)



Reactor for acetic anhydride pilot plant [FreeCAD file]:

reactor for acetic anhydride.FCStd

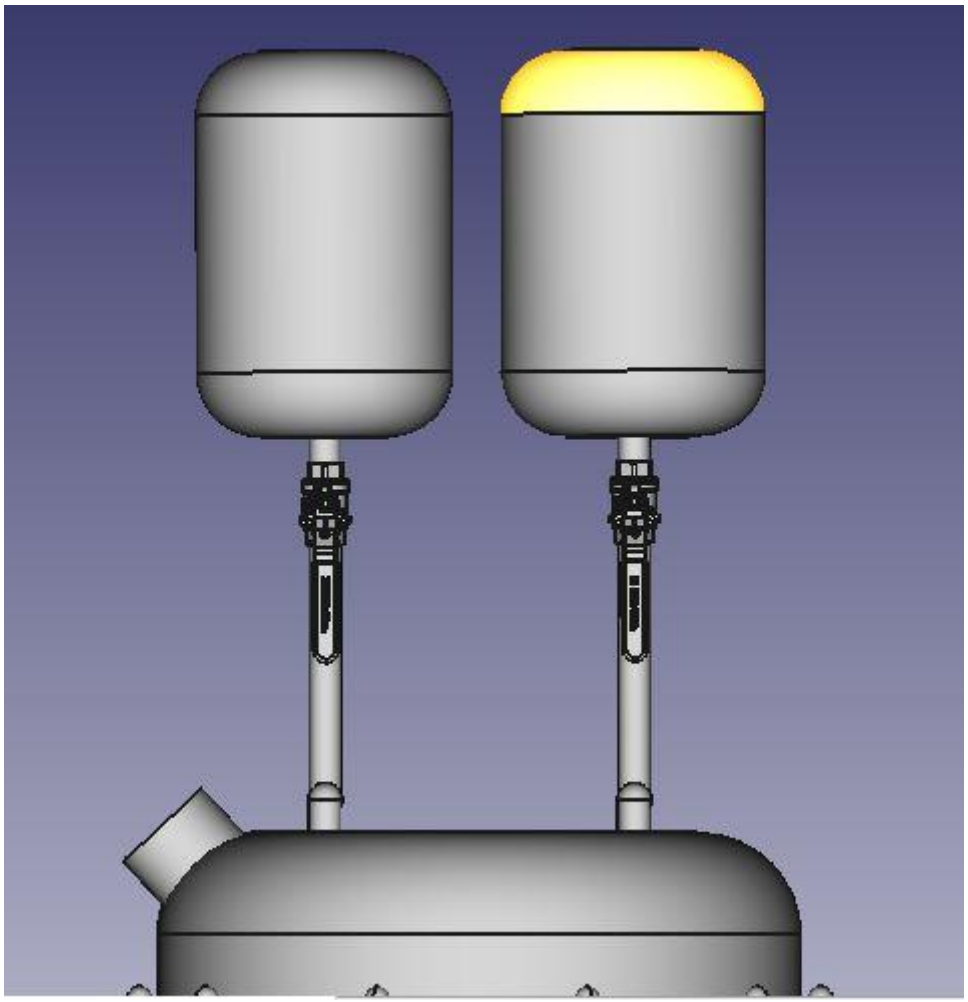


Condenser for acetic anhydride pilot plant [FreeCAD file]:

25122023_Condenser.FCStd

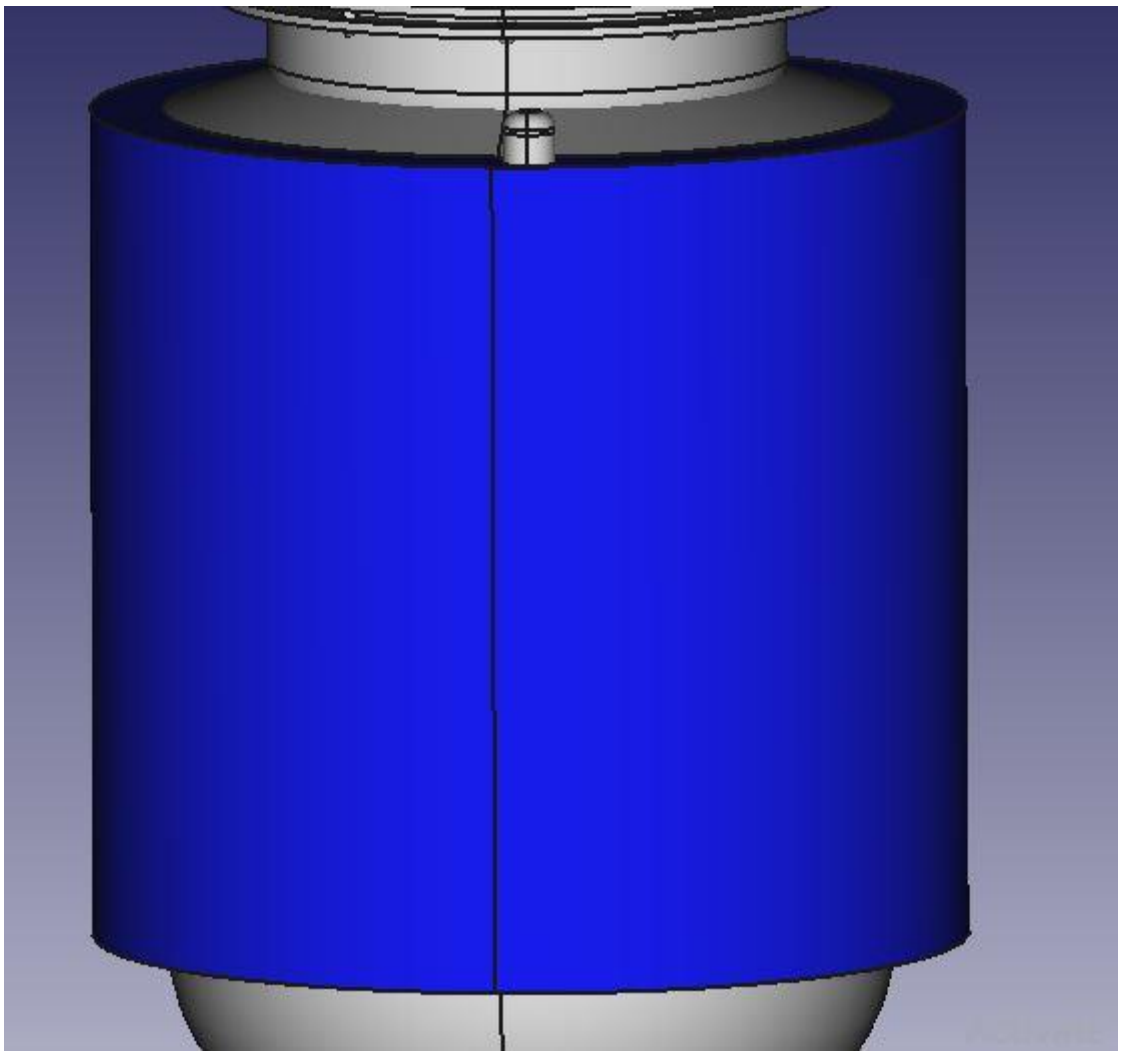
Mixer for acetic anhydride pilot plant [FreeCAD file]:

agitator 3.FCStd



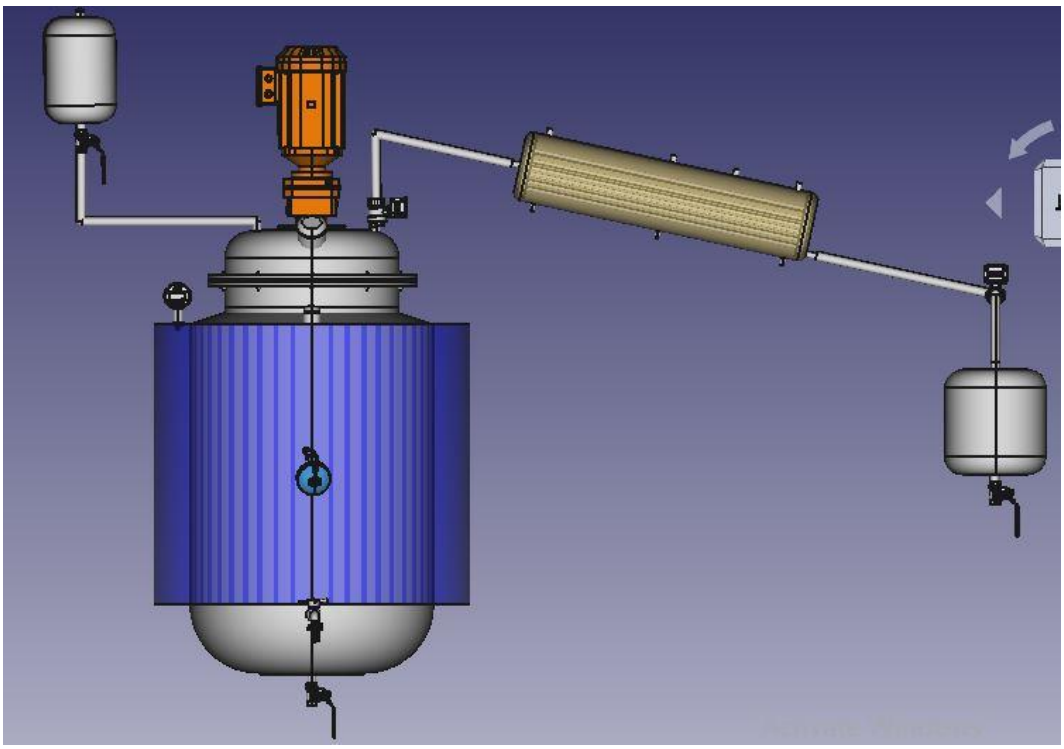
External tanks for acetic anhydride pilot plant

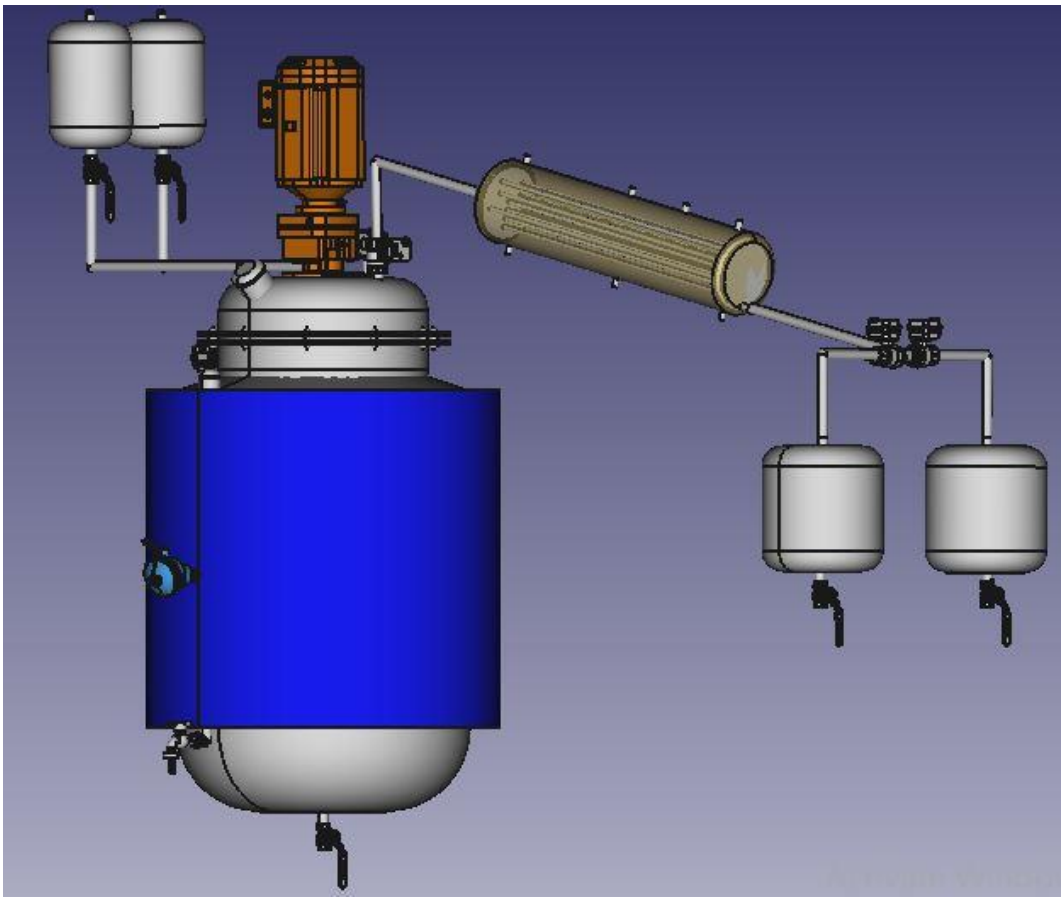
reactor for acetic anhydride0.FCStd



Jacketed reactor for acetic anhydride pilot plant [FreeCAD file]:

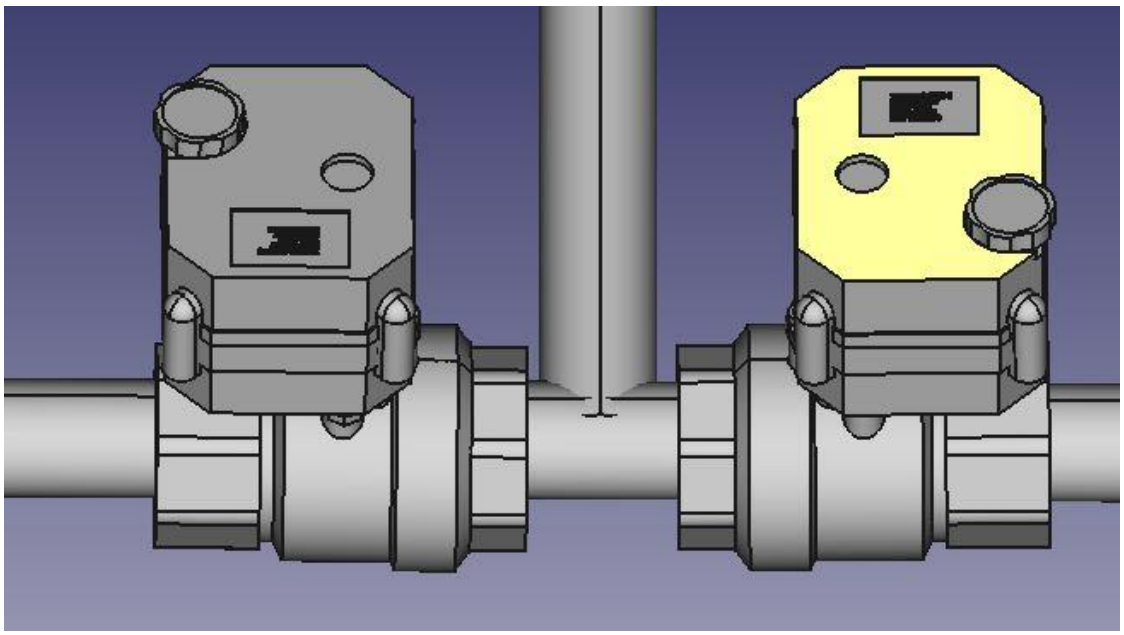
reactor for acetic anhydride1.FCStd



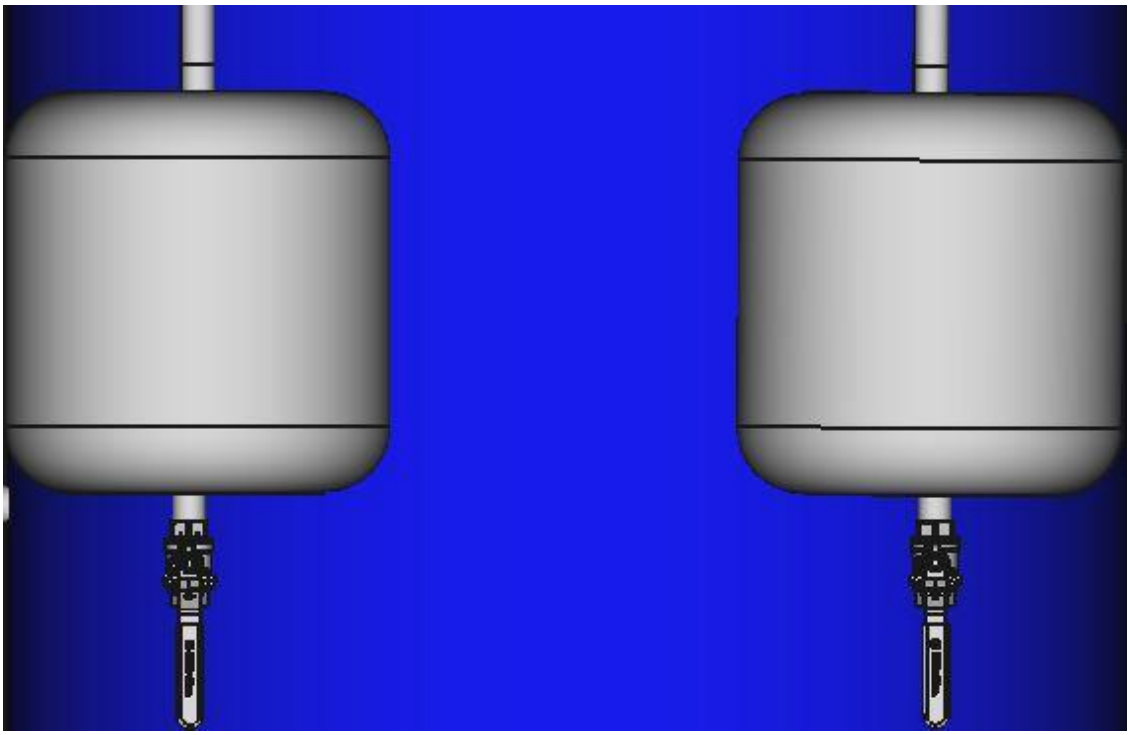


Pilot Plant for Acetic Anhydride [FreeCAD file]:

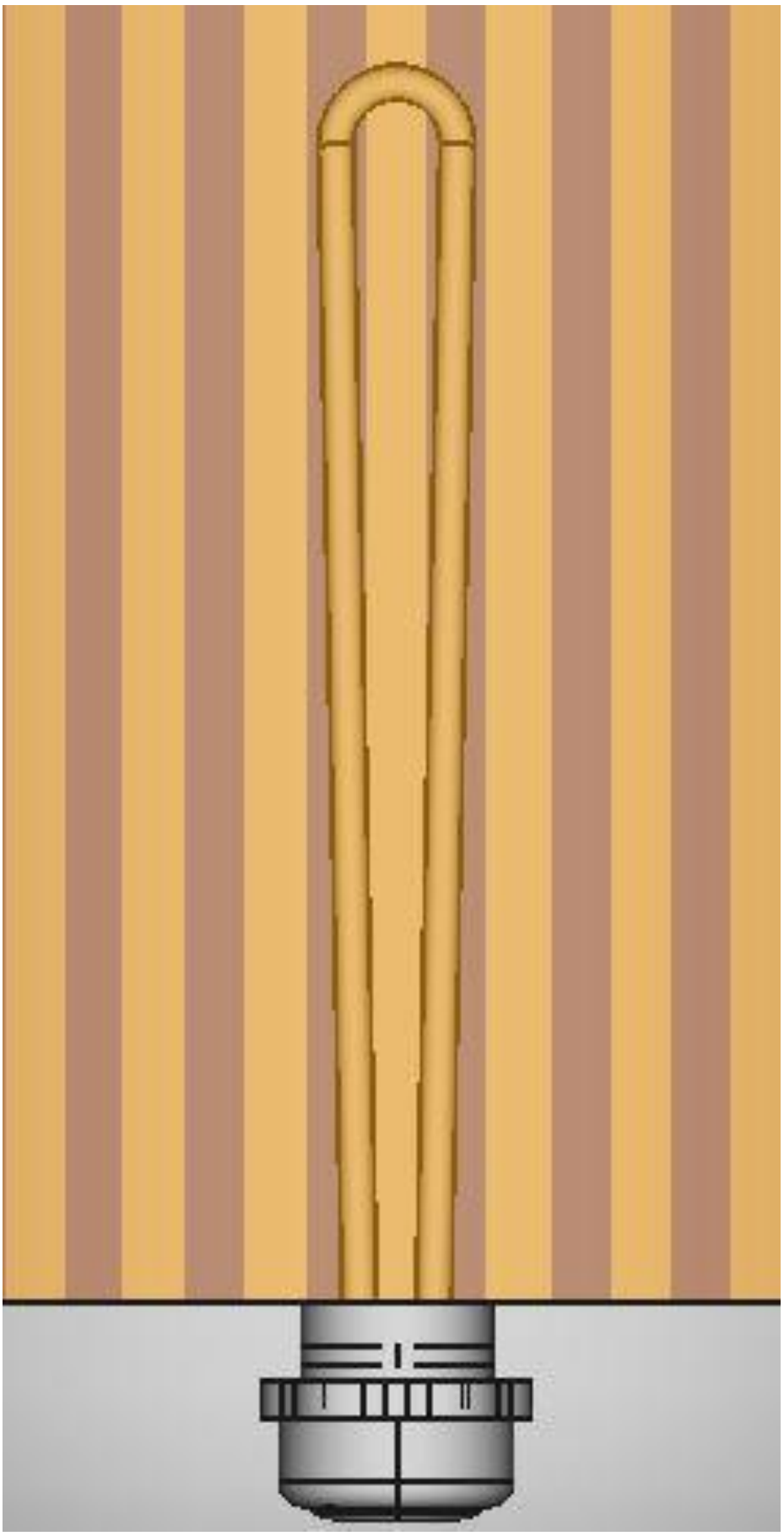
Pilot Plant for acetic anhydride productic



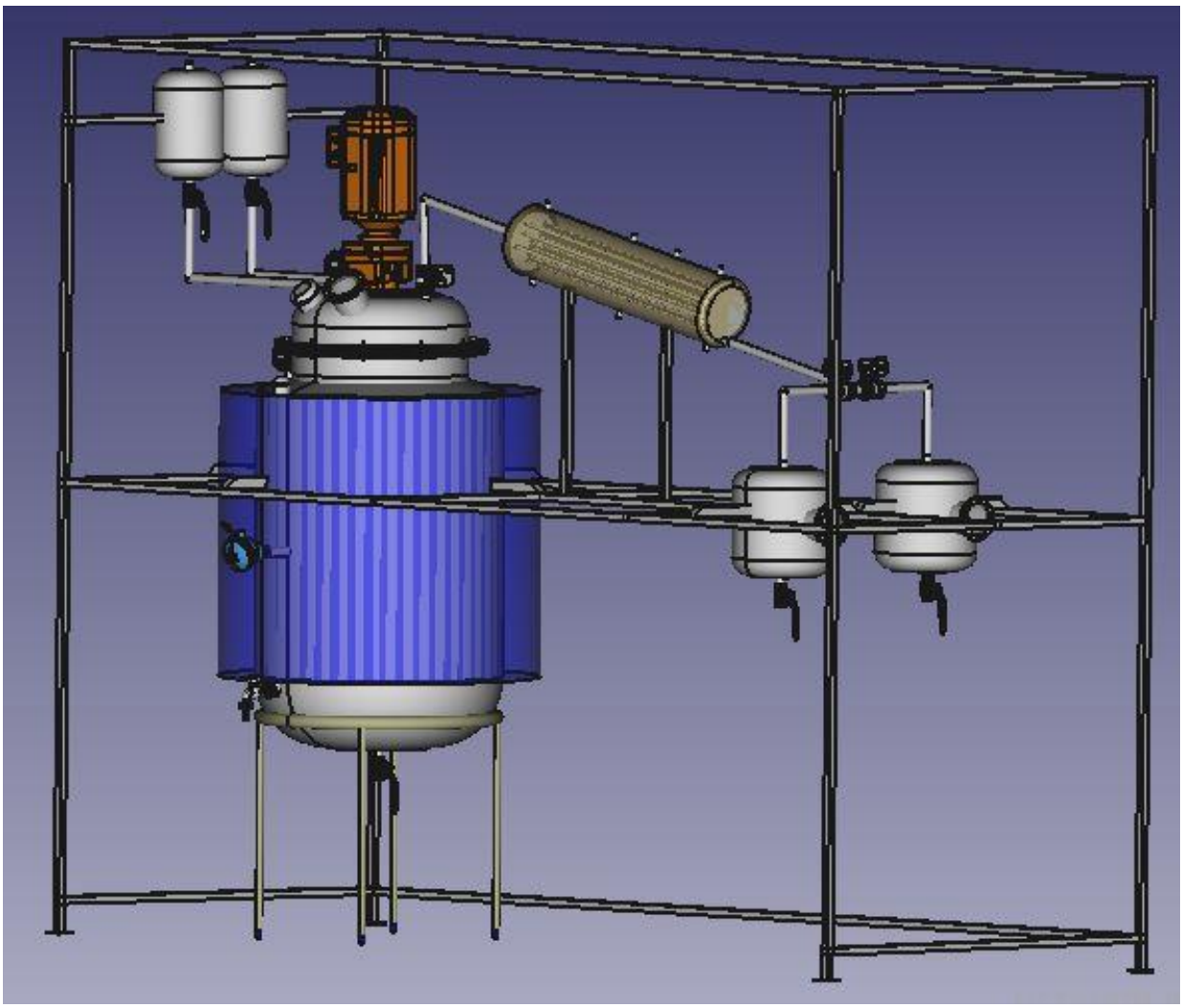
Electrical valve for Acetic Anhydride Pilot Plant



Products tanks (acetic anhydride tank and solvent tank) for Acetic Anhydride Pilot Plant



Electrical Heater for Acetic Anhydride Pilot Plant



Stand for Acetic Anhydride Pilot Plant - 07.02.2024 [FreeCAD file]:

reactor for acetic anhydride2.FCStd

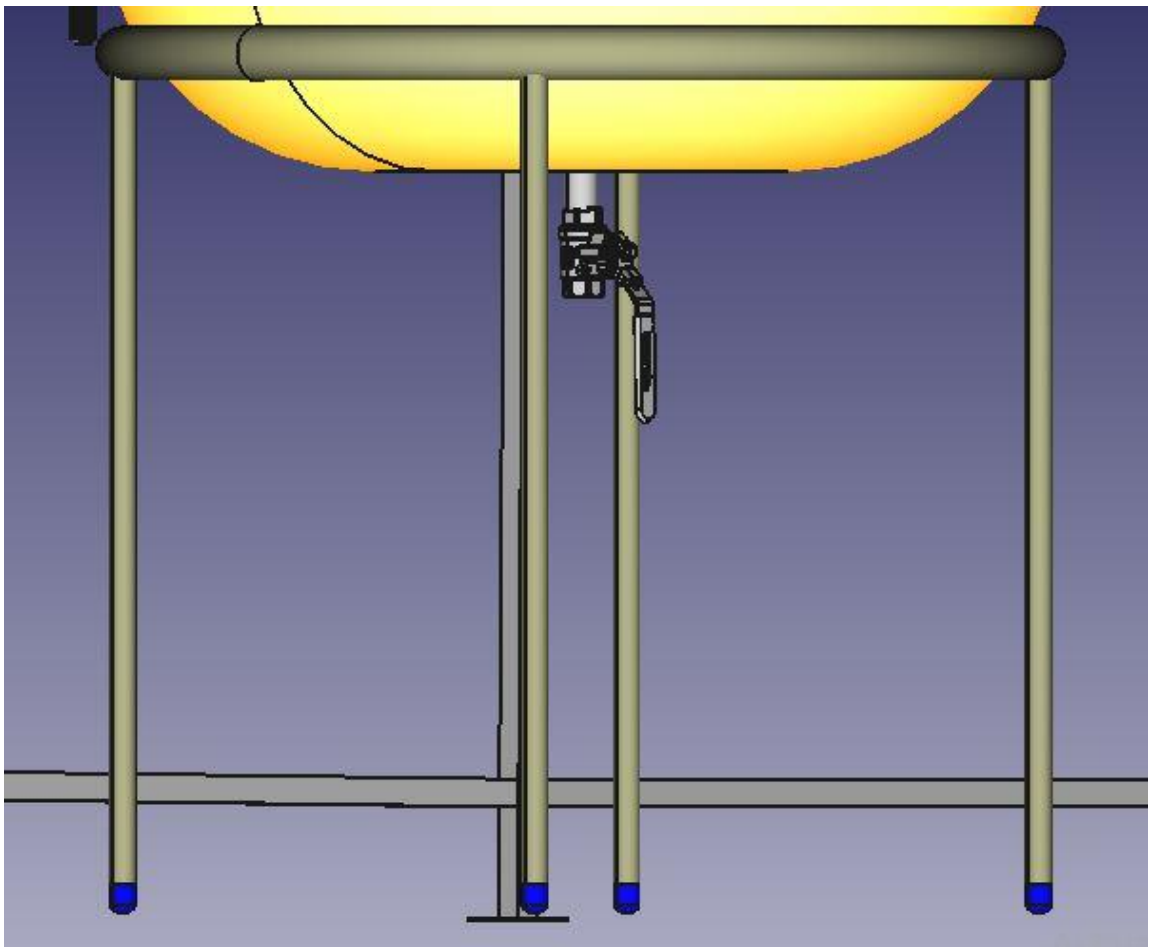


Image 4: Support Stand for the "Reactor of Acetic Anhydride" 07.02.2024

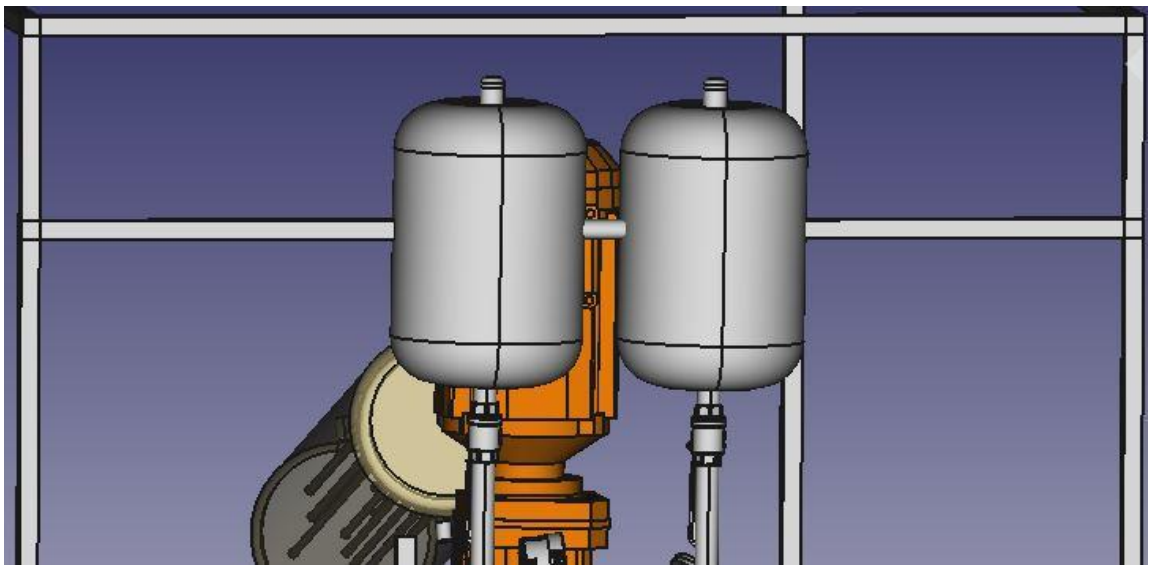


Image 5: support for 2 reagents tanks 07.02.2024

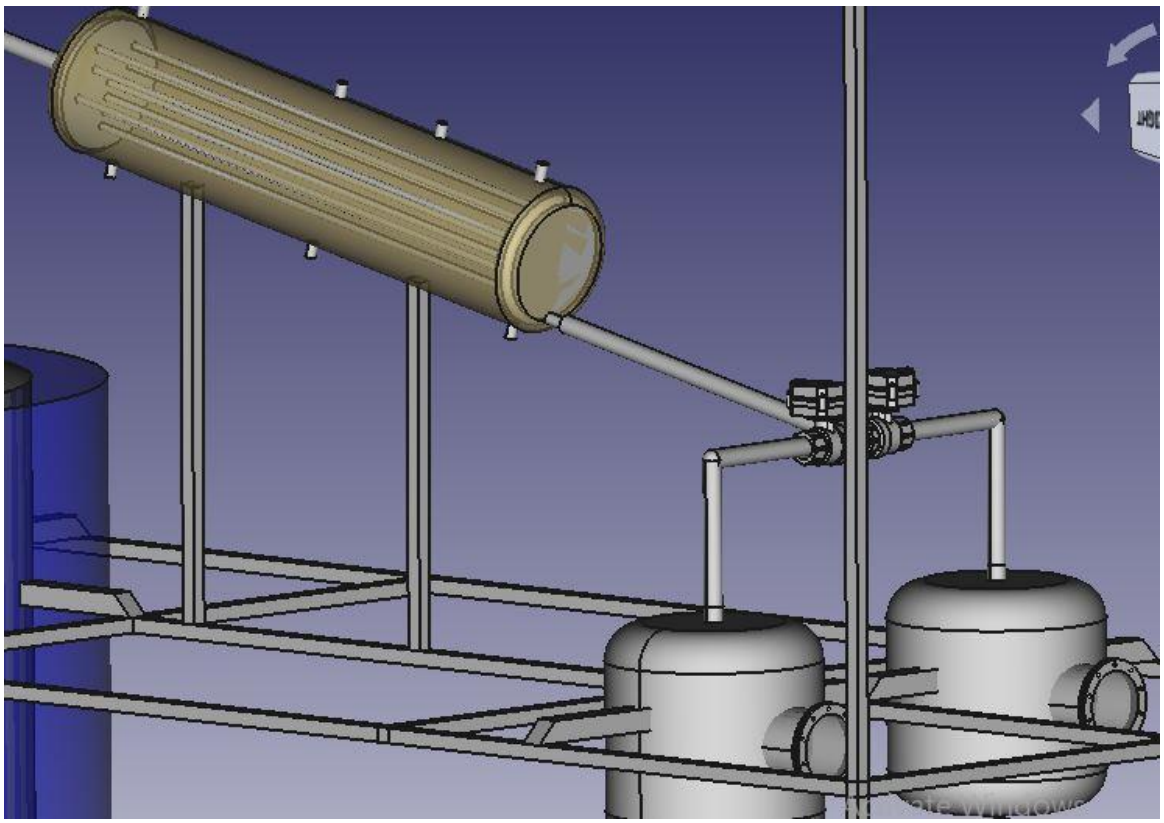
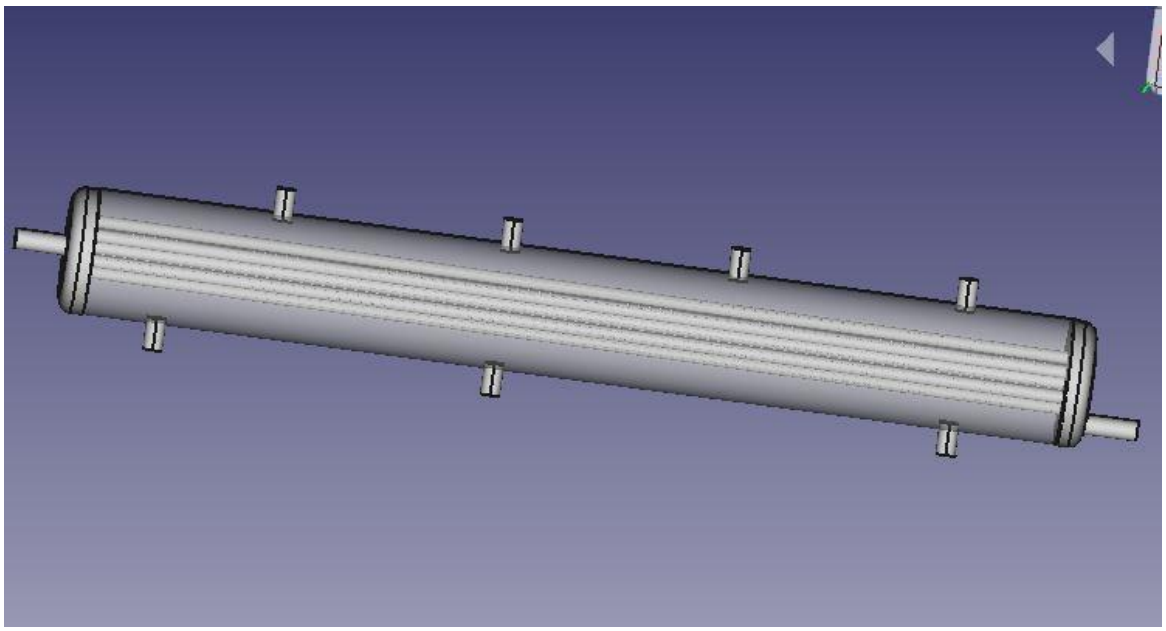


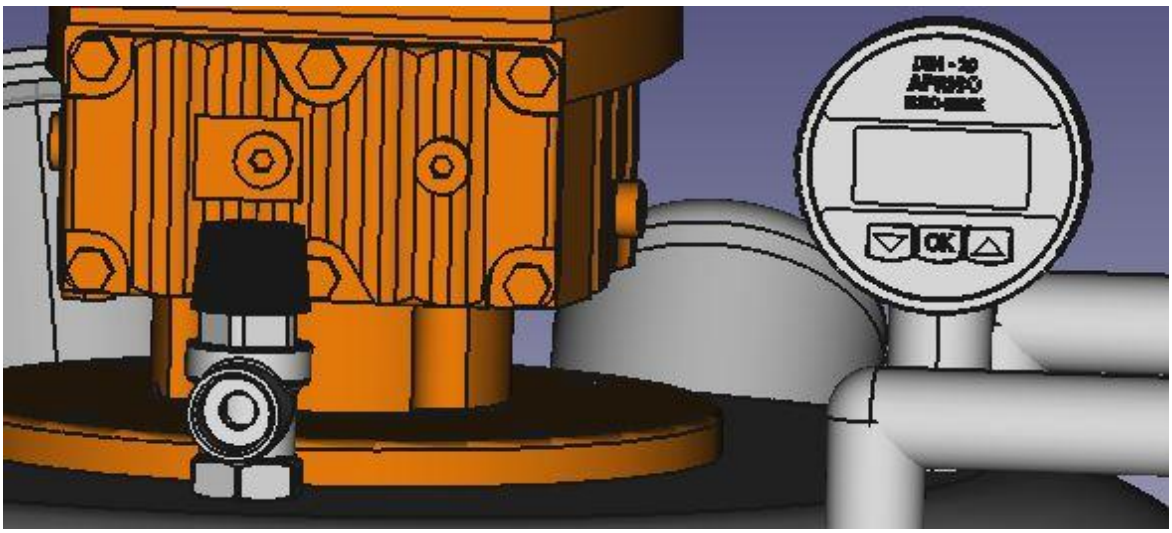
Image 6: Support for 2 products tanks and condenser 07.02.2024



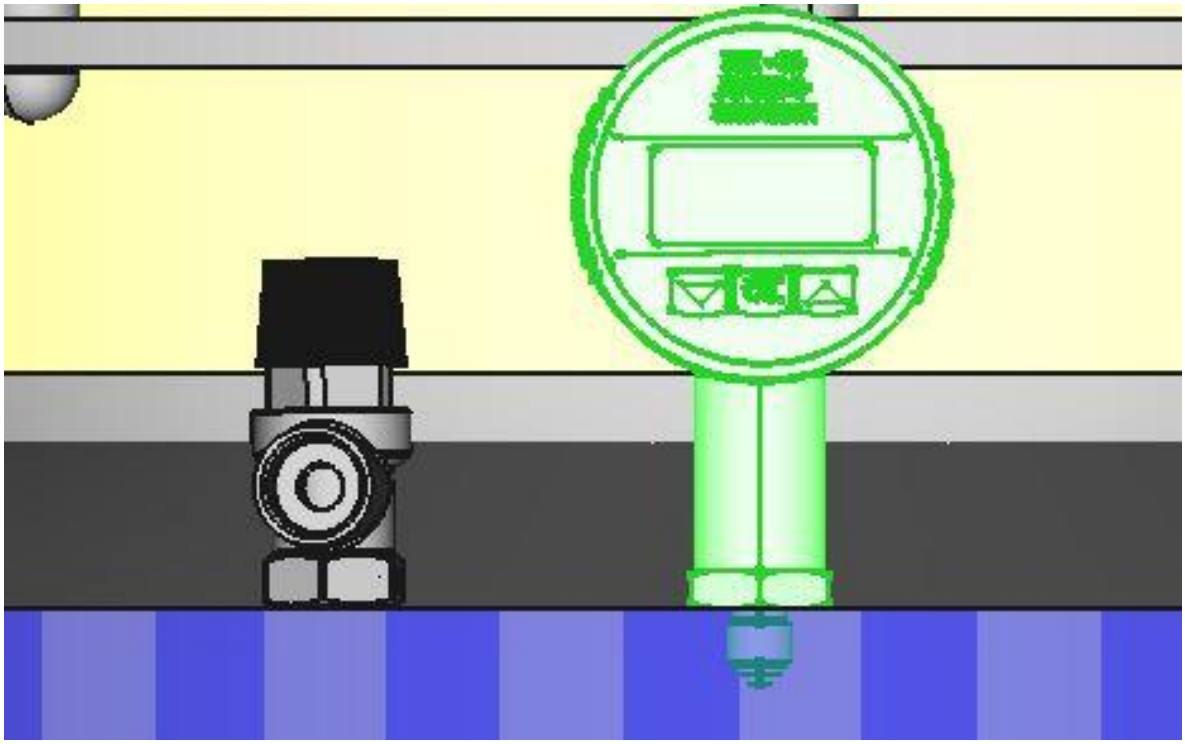
New condenser for acetic anhydride pilot plant 12.02.2024

Last update condenser acetic anhydride pilot plant" 12.02.2024 [FreeCAD file]:

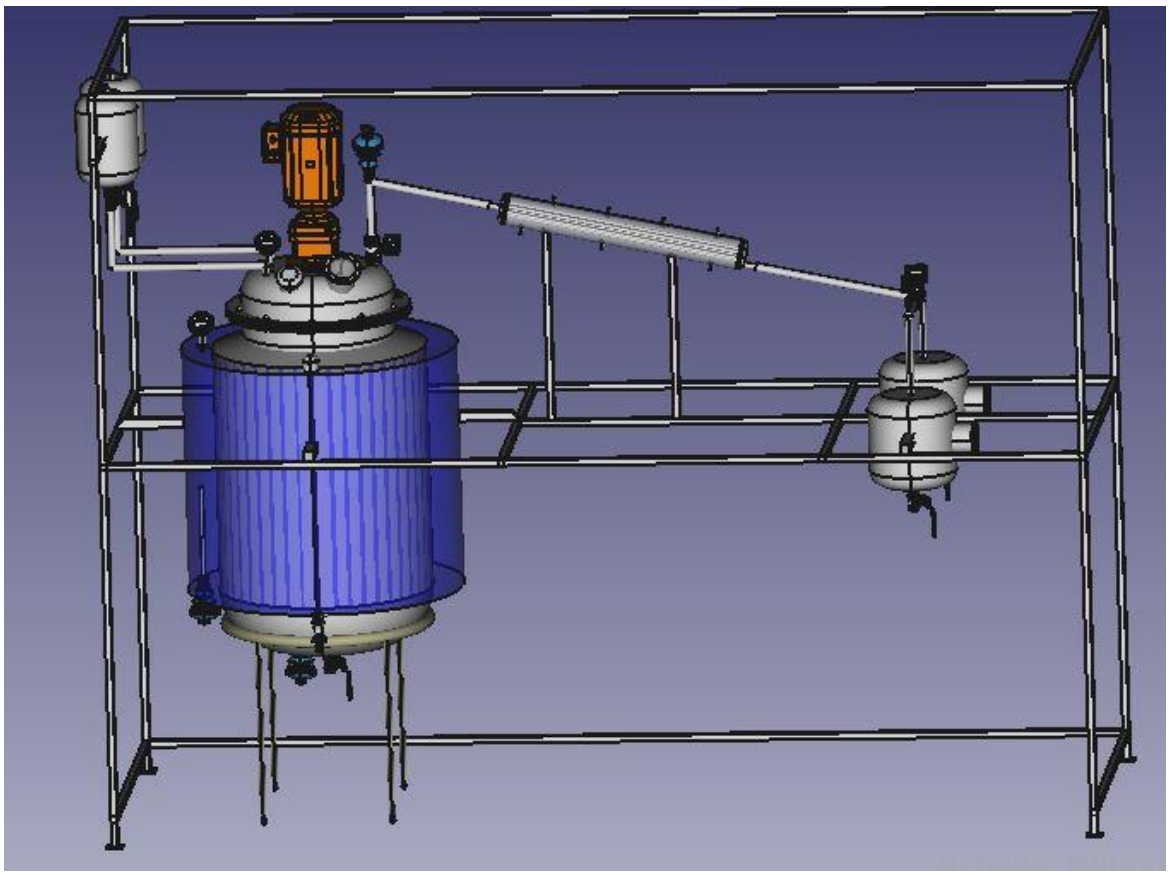
Condenser
MK.FCStd



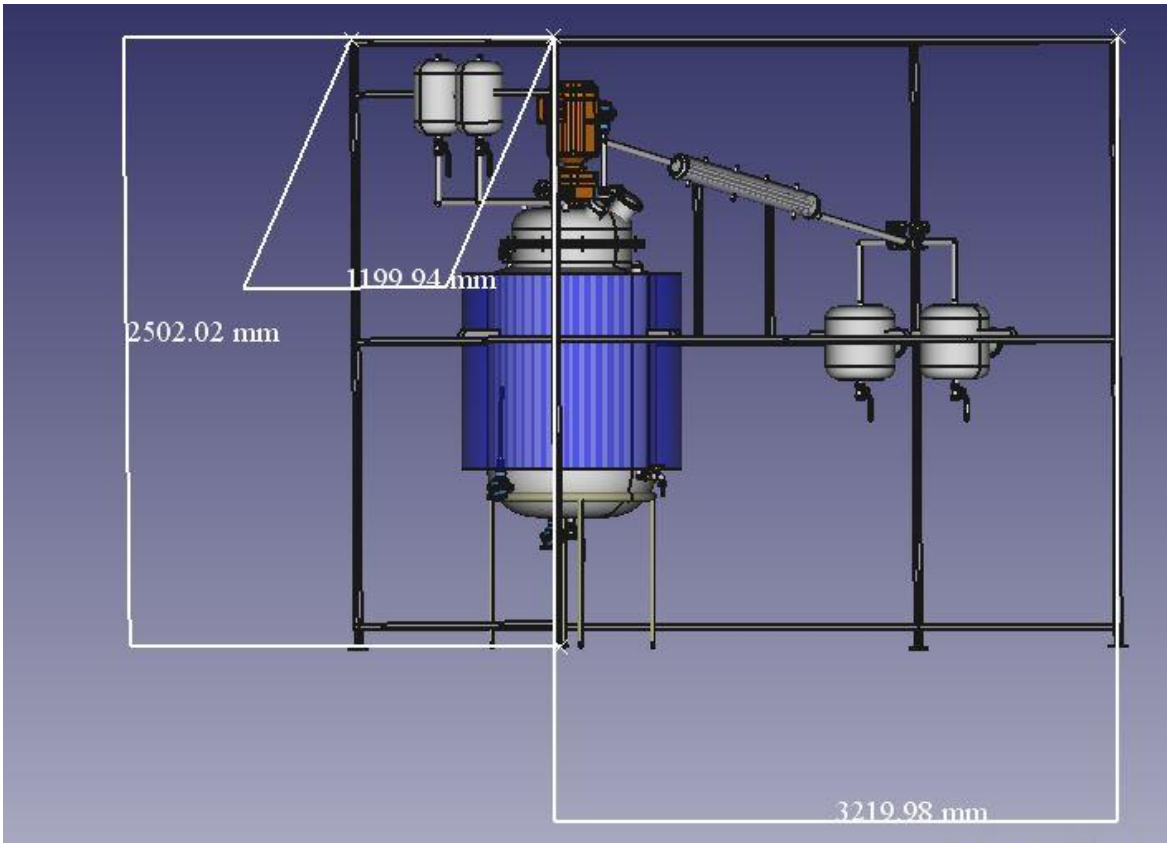
Safety pressure valve in reactor for acetic anhydride pilot plant 22.02.2024

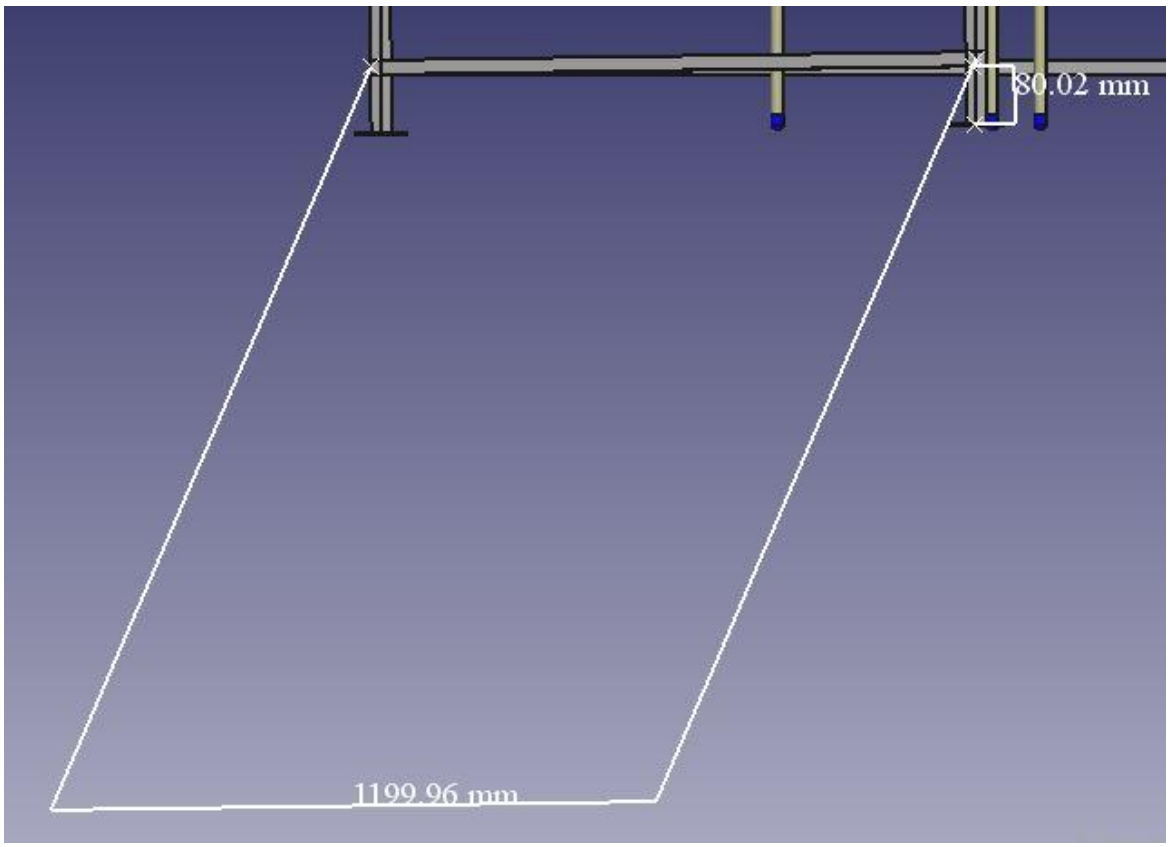


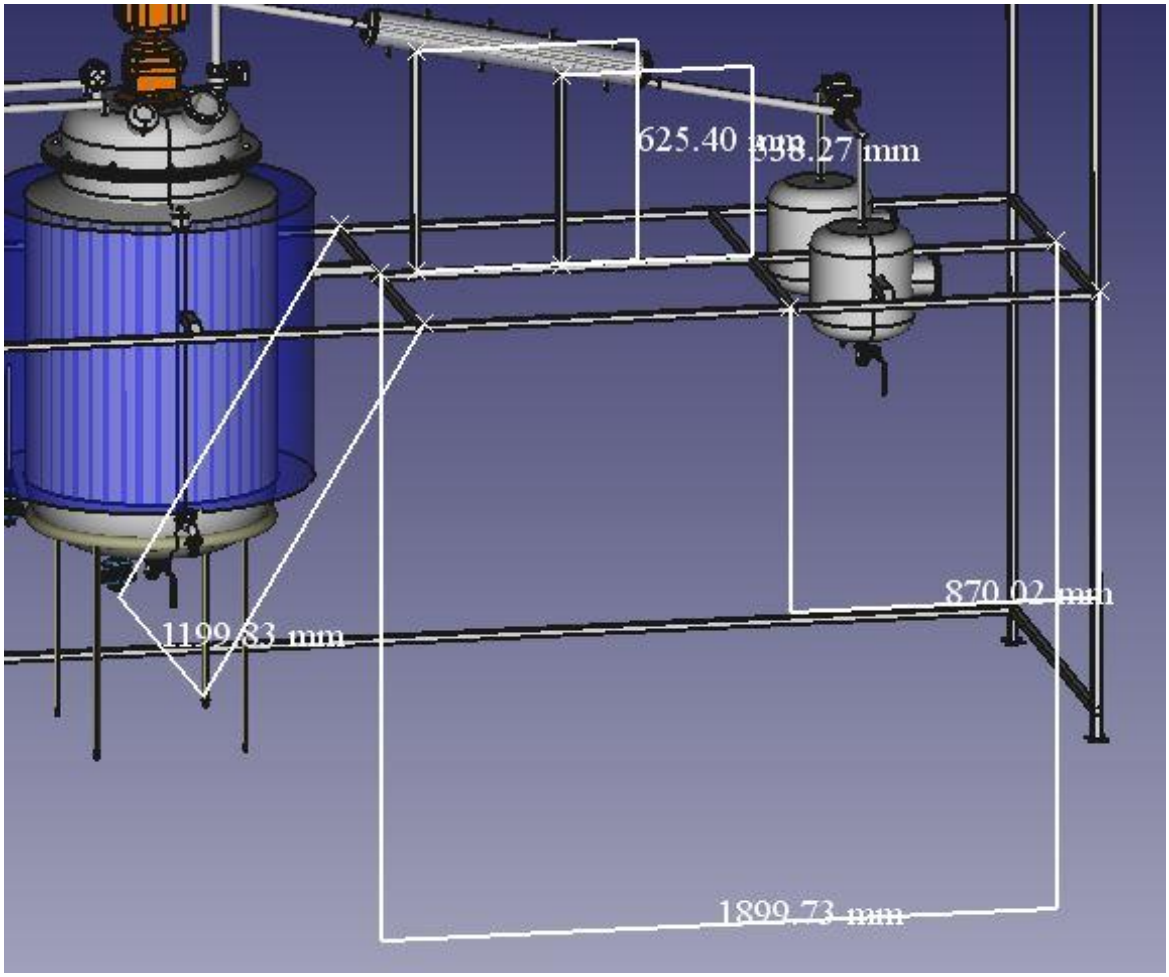
Safety pressure valve in jacket for acetic anhydride pilot plant 22.02.2024

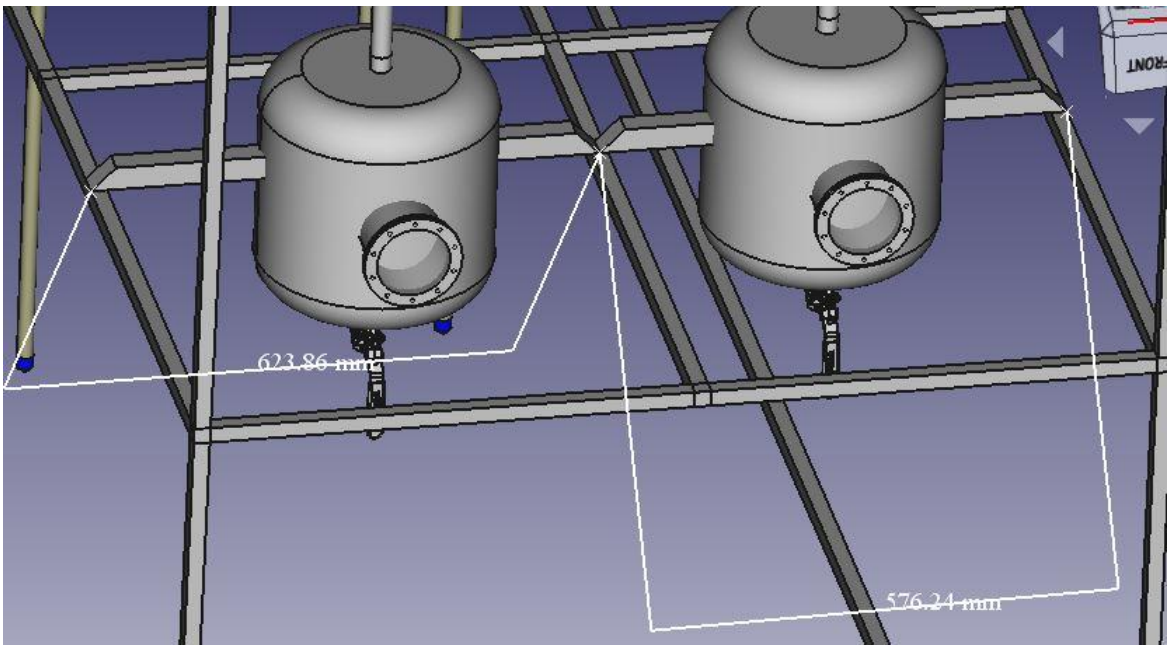


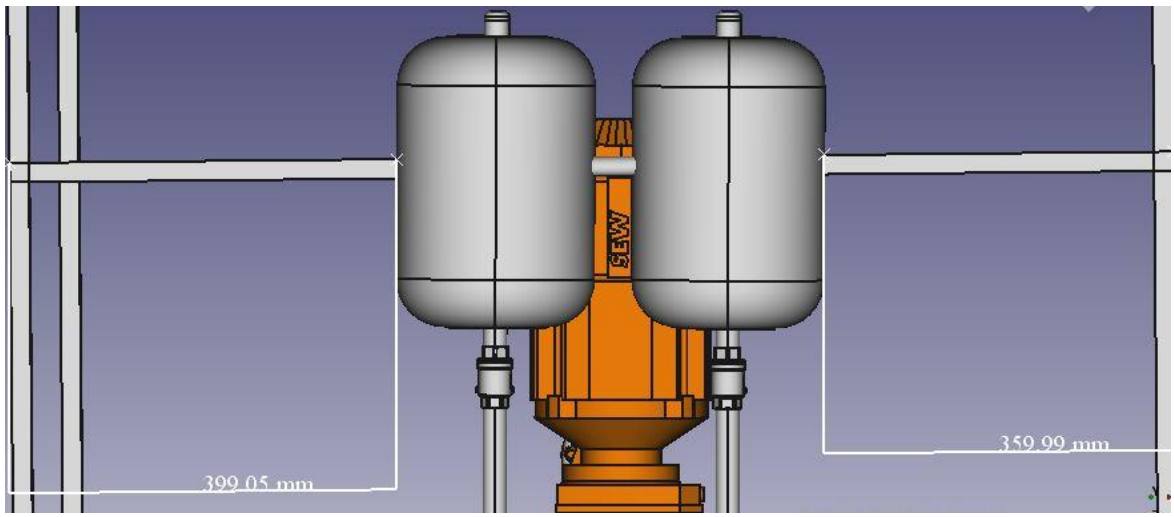
Measurements from the Acetic Anhydride pilot plant

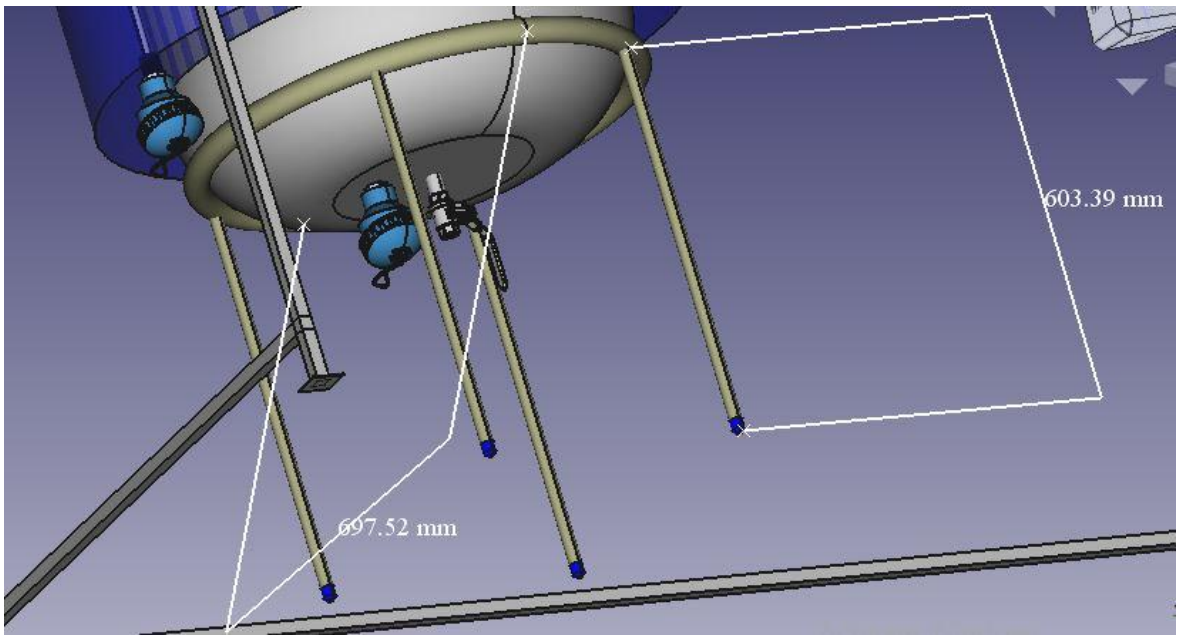


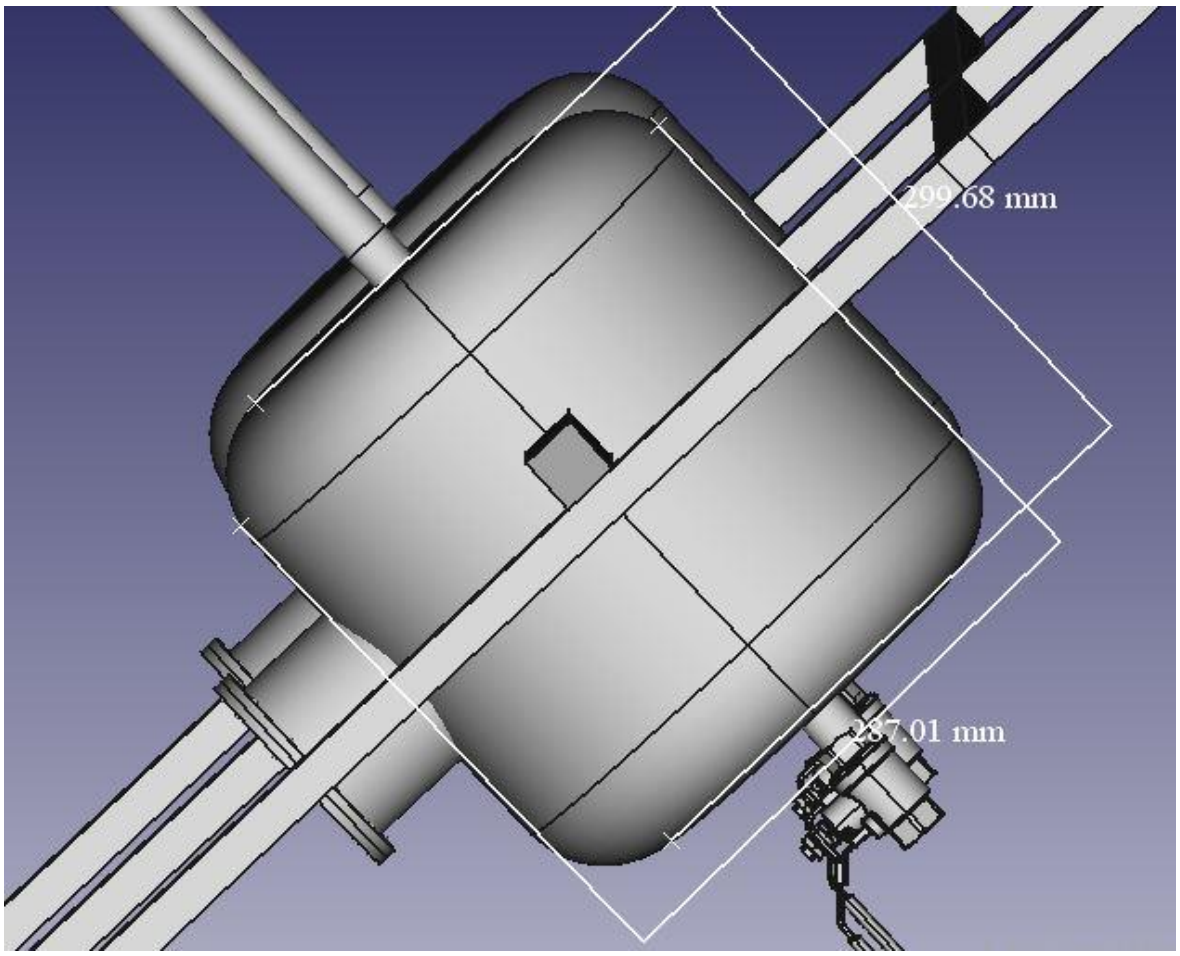

80.02 mm
1199.96 mm

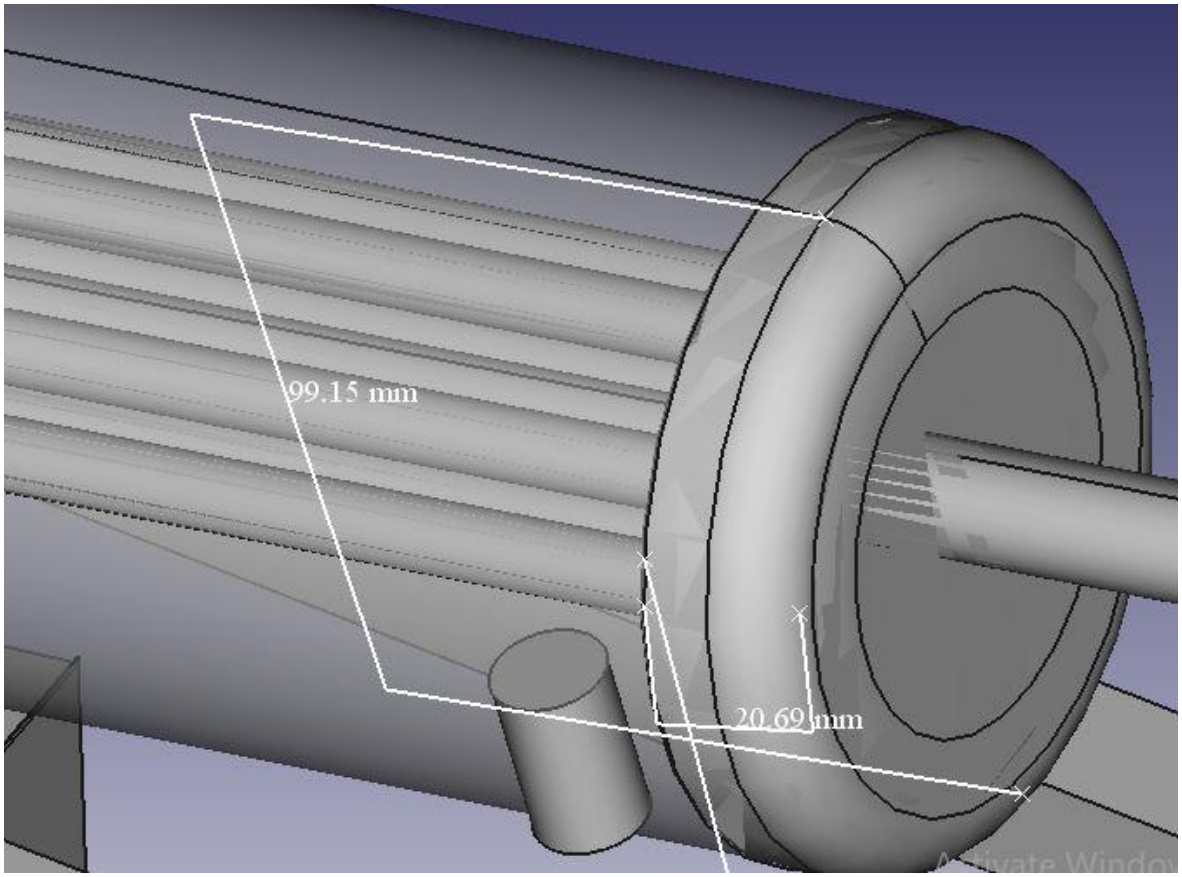

625.40 mm
870.02 mm
1199.83 mm
1899.73 mm

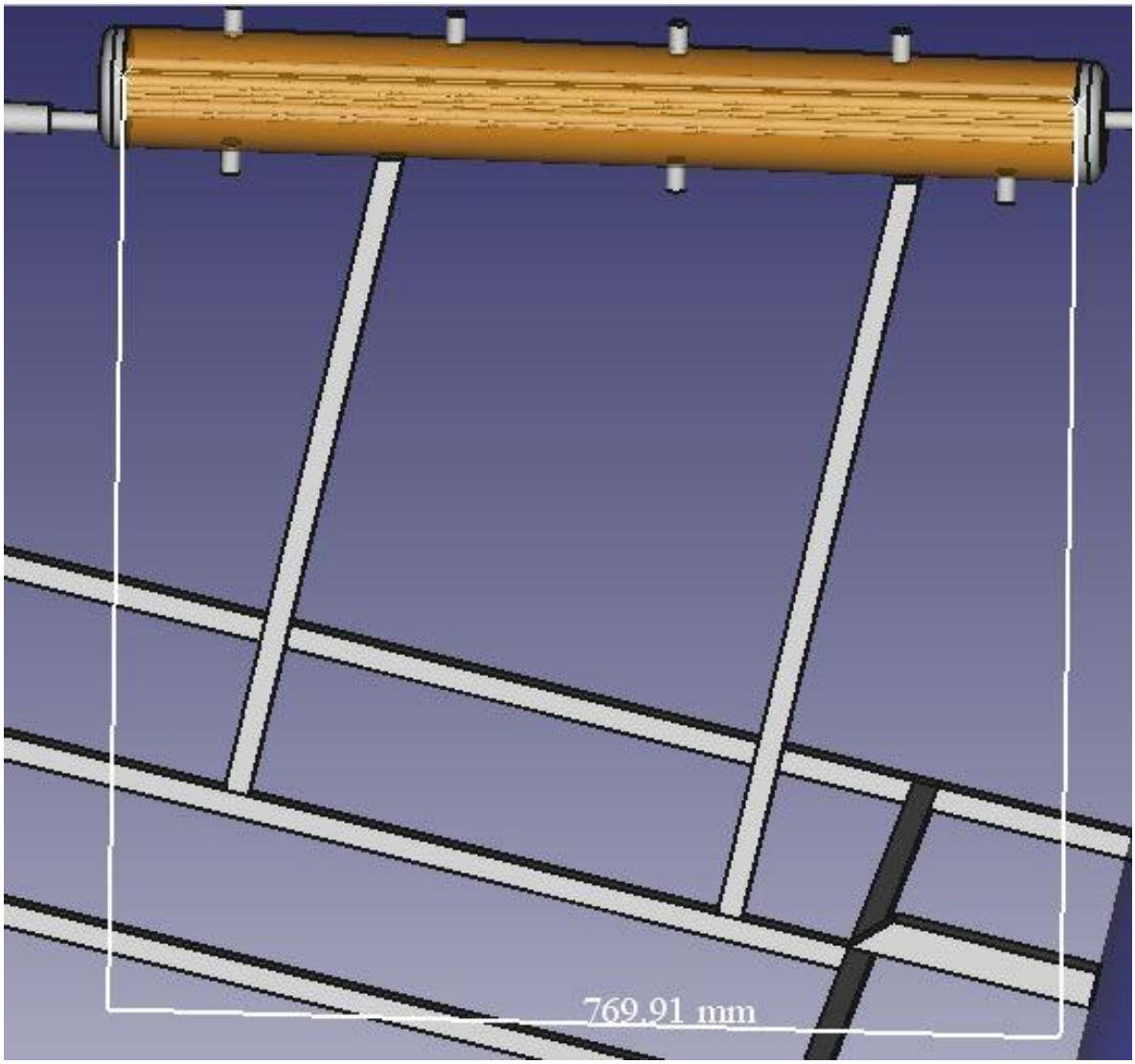

623.86 mm
576.24 mm

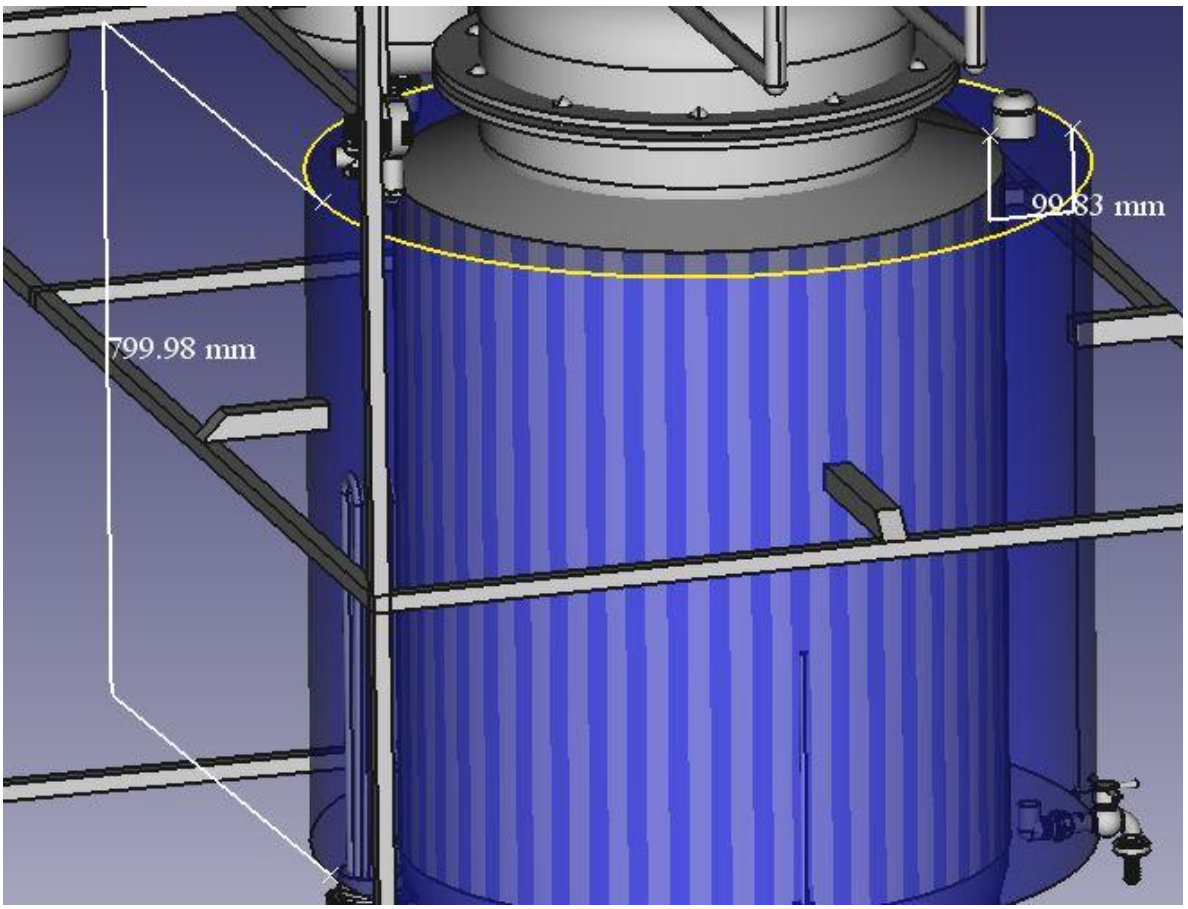

SEW
399.05 mm
359.99 mm


603.39 mm
697.52 mm


299.68 mm
237.01 mm


99.15 mm
20.69 mm


769.91 mm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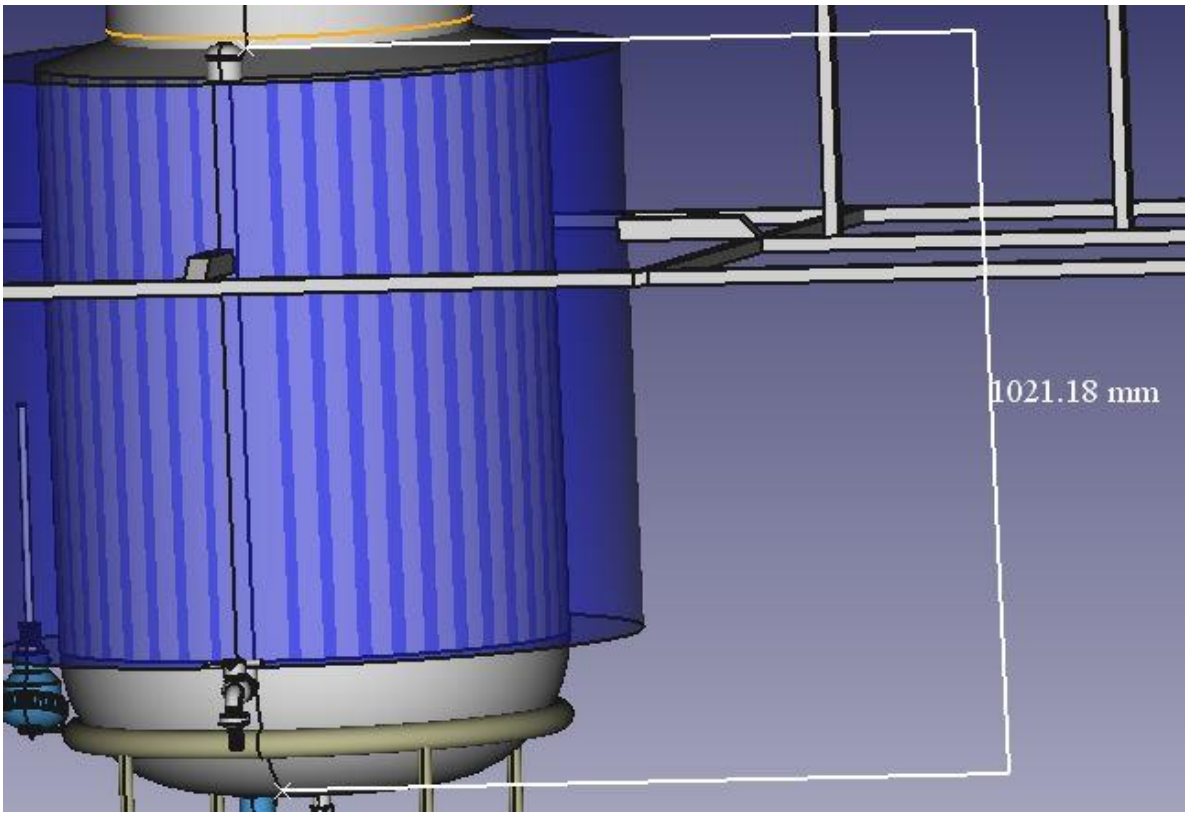
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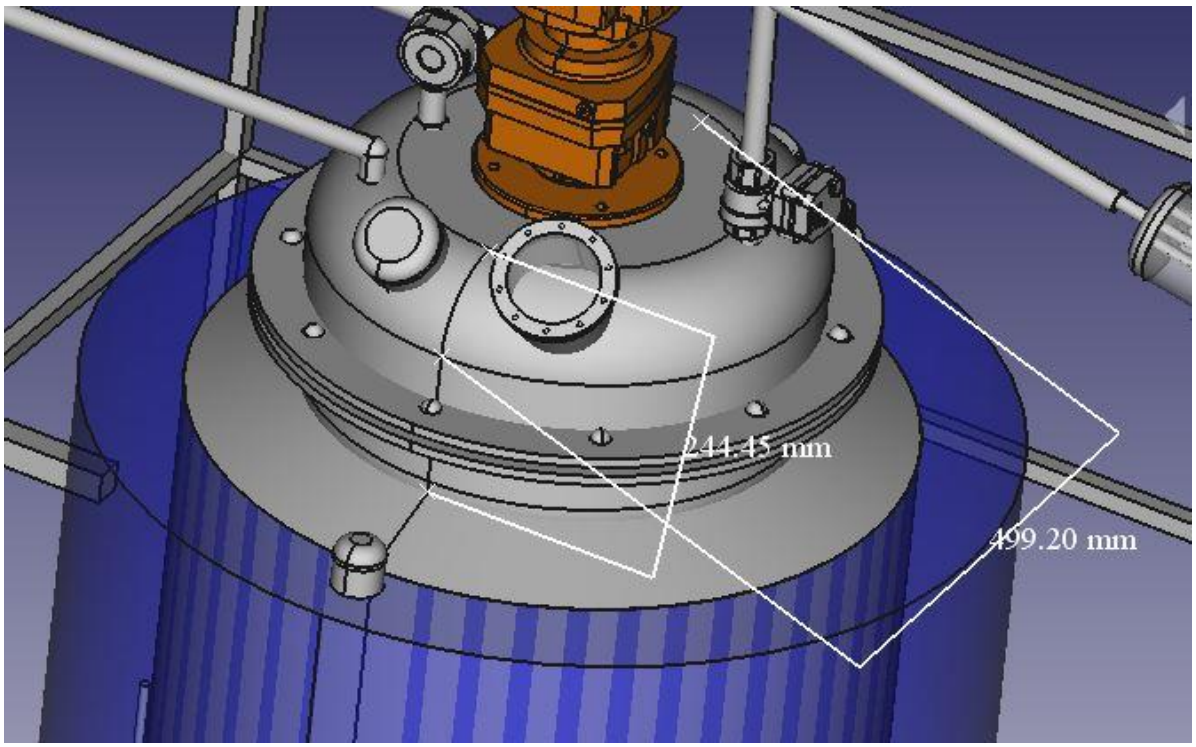
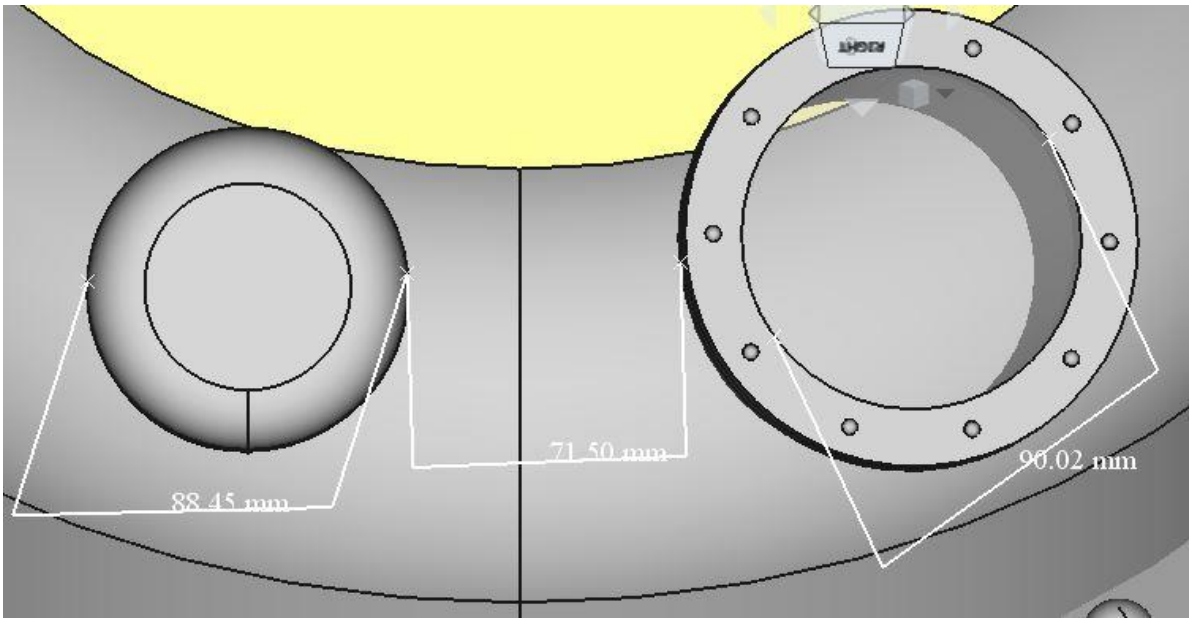
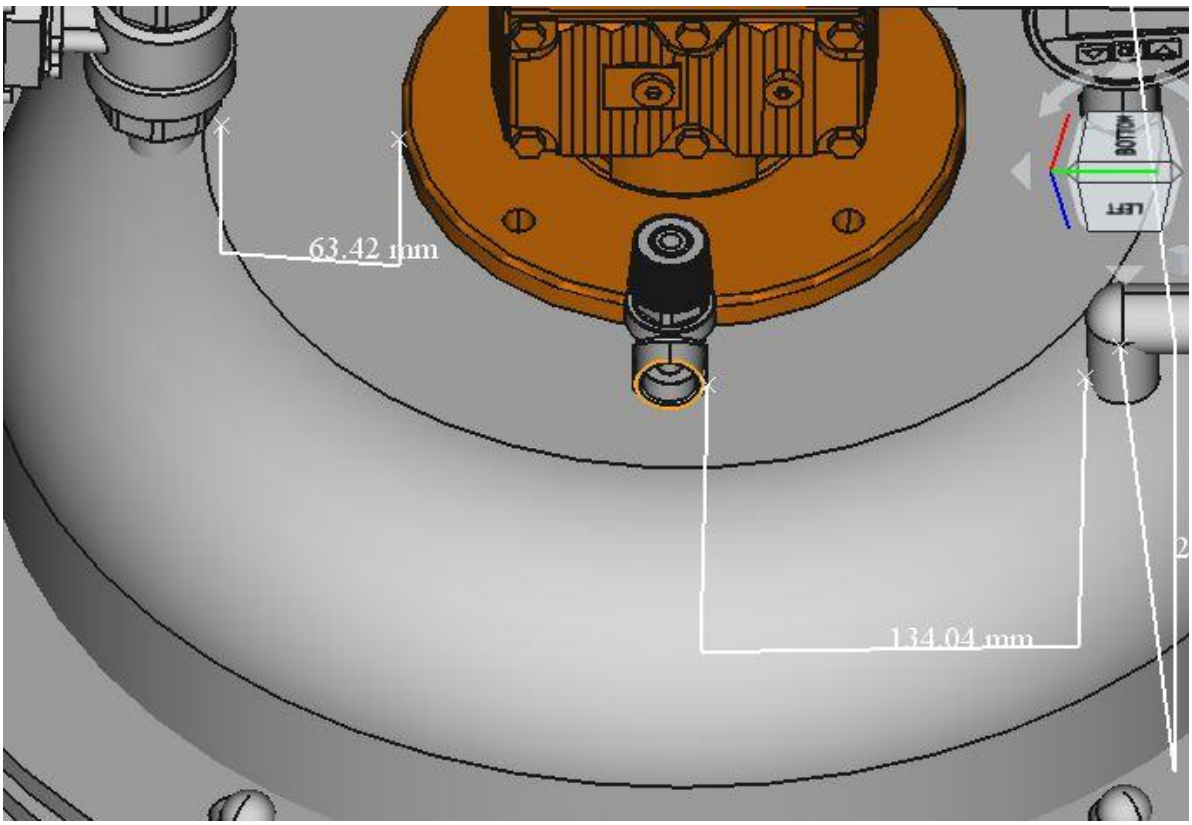
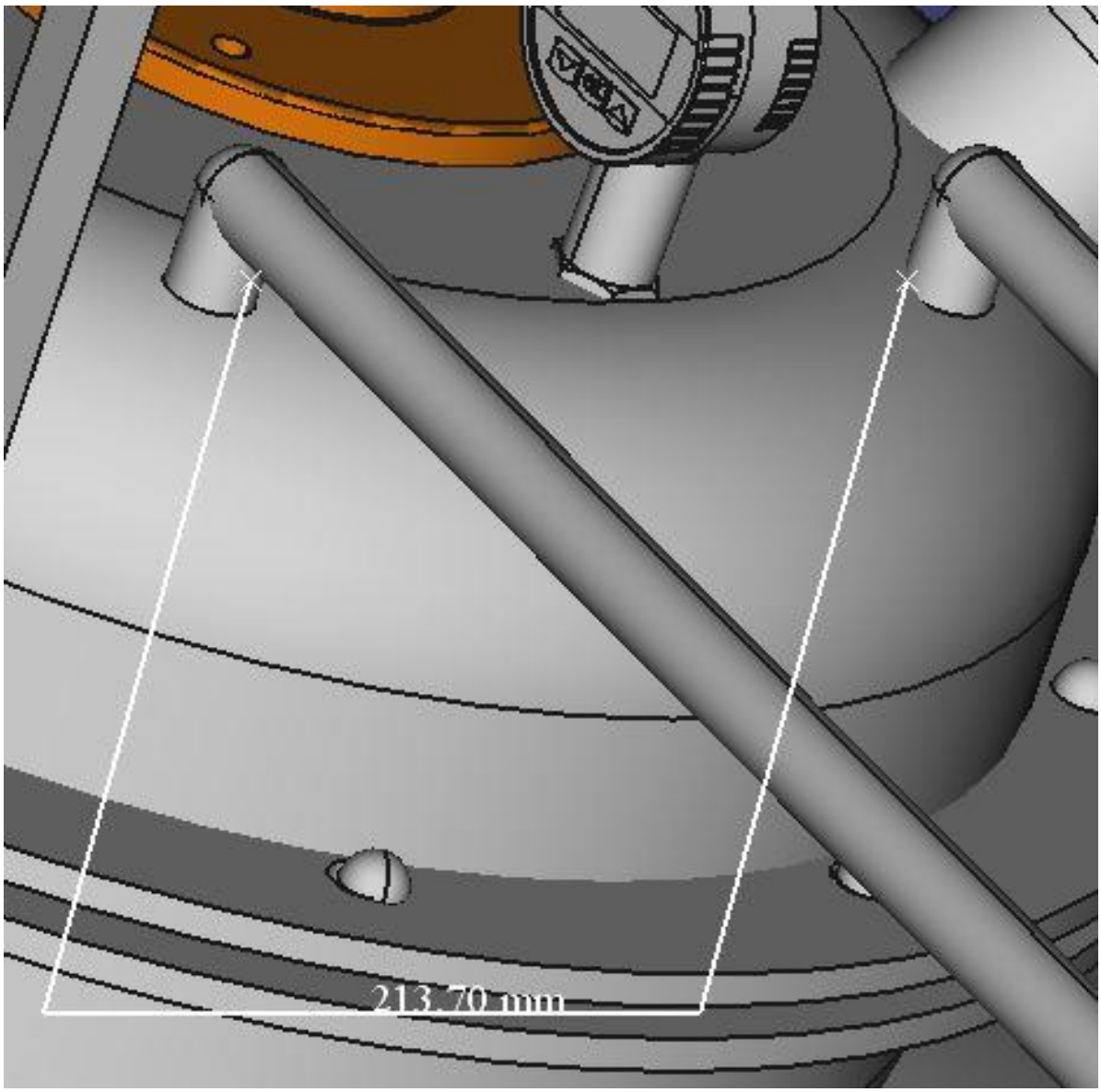
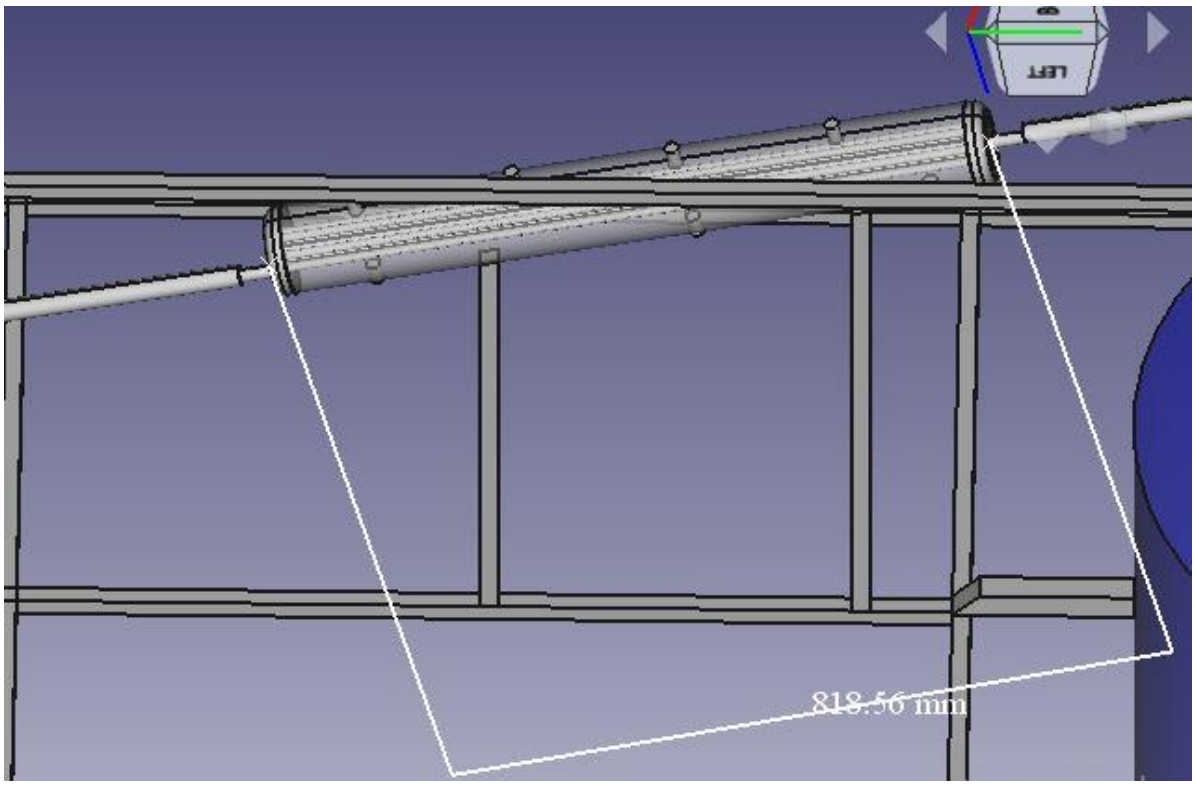
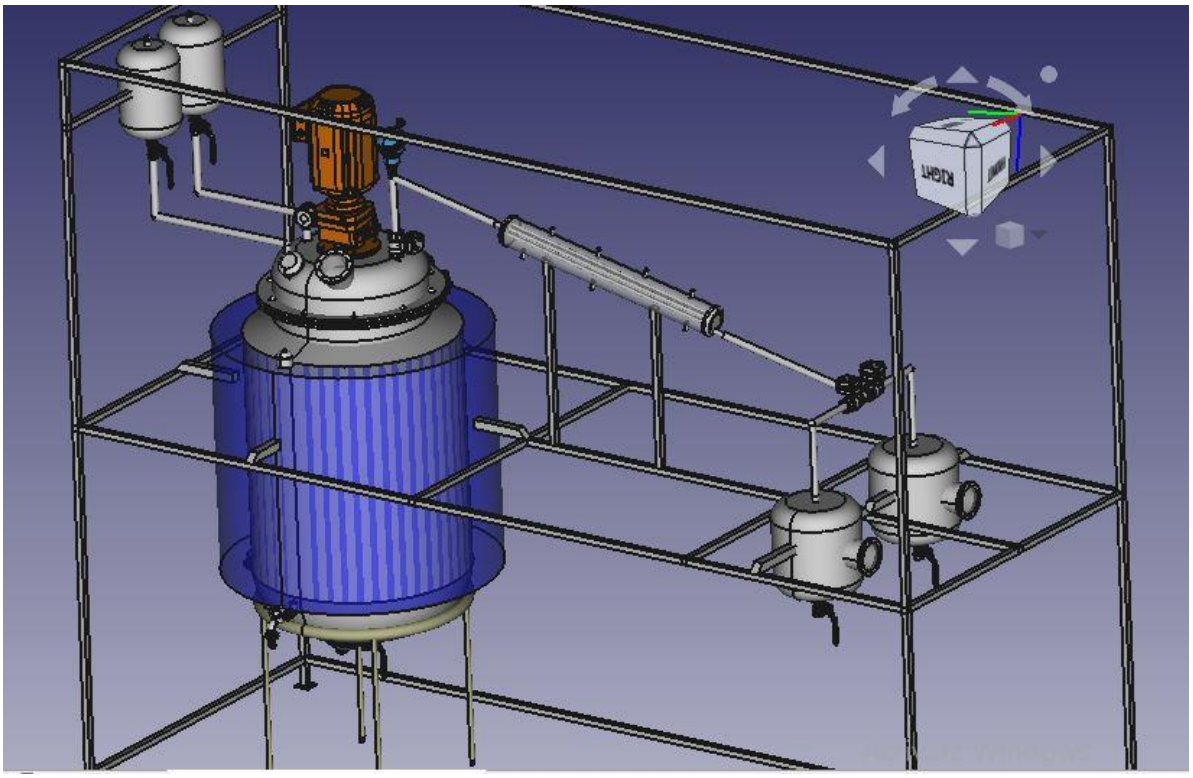

244.45 mm
499.20 mm

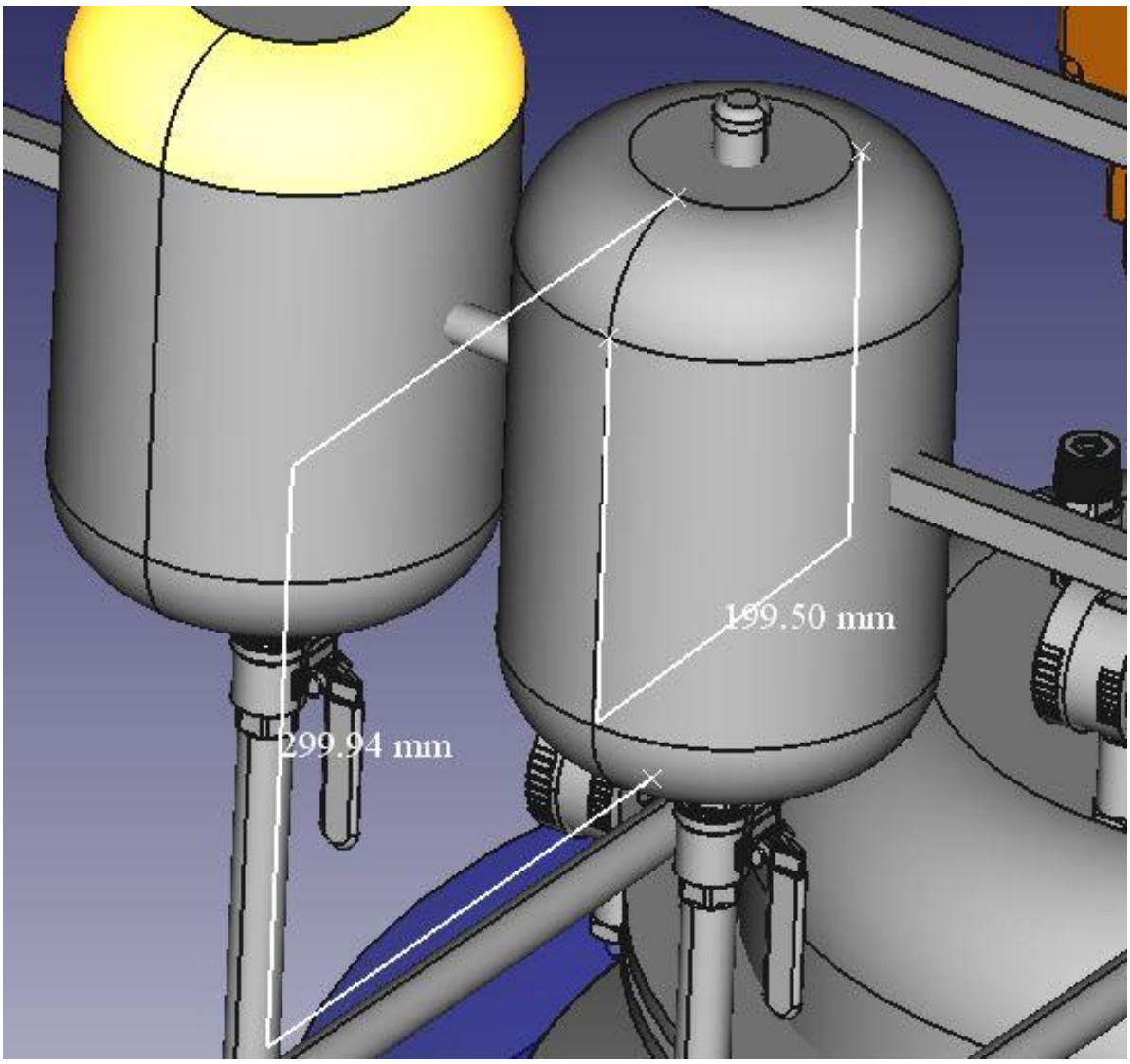

71.50 mm
88.45 mm
90.02 mm


63.42 mm
134.04 mm
BOTTOM
LEFT


213.70 mm


LEFT
818.56 mm


RIGHT



21.3.2 Acetic anhydride PCS implementation

- PCS_AceticAnhydrideProduction_250225 - GUI


Acetic
Anhydride_PCS_GUI


Acetic
Anhydride_PCS_GUI

- PCS_AceticAnhydrideProduction_250225 - GUI


Acetic
Anhydride_PCS_Con

- Acetic Anhydride PCS _PLC Modbus addresses_201224


Acetic Anhydride
PCS _PLC Modbus ac

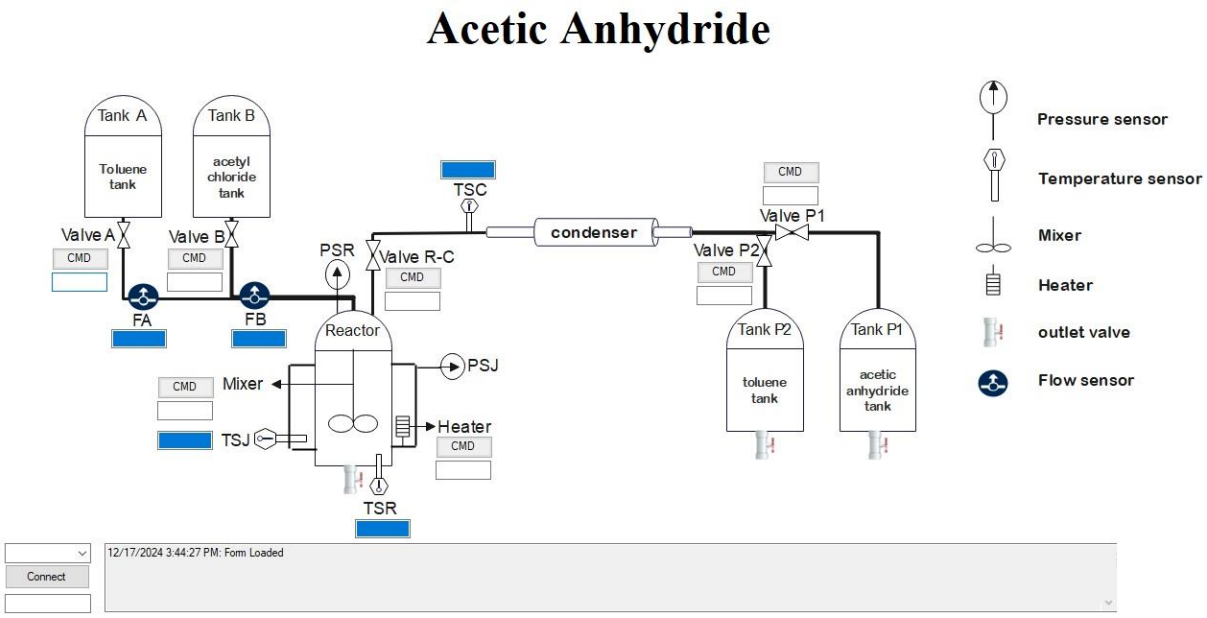
- Acetic_Anhydride_PCS_PLC_24.12.24

Acetic_Anhydride_P
CS_PLC_24.12.24.dvp

- All files concerning the process control system

PCS_AceticAnhydrideProduction_250225.zip

Graphical user interface



21.4 Requirements For Acetic Anhydride Pilot Plant Production

21.4.1 System requirements

- Acetic Anhydride Pilot Plant shall be able to produce the Acetic anhydride.
- The control panel shall be able to control all valves, mixer and read the data of the sensors (Temperature-pressure-Heater).

21.4.2 Physical requirements

- The pipes shall be able to withstand the temperatures and pressures that exist at the points.
  - Temperature that shall be withstood: +100°C.
  - Pressure that shall be withstood: 2 bars.
- The tanks shall be able to withstand the temperature exchanges, pressures, and mechanical forces that exist at the points.
  - Temperature that shall be withstood: +100°C.
  - Pressure that shall be withstood: 2 bars
  - mechanical force: mixer movements and rotation.

- The sensors (Temperature, Pressure, and Flow) shall be able to withstand the temperatures and pressures that exist at the points.
  - Temperature that shall be withstood: +100℃.
  - Pressure that shall be withstood: 2 bars.

21.4.3 Chemical requirements

- The Tanks system shall be able to insulate the chemical reagents.
- The Tanks system shall be able to withstand the corrosion with organic reagents acetic anhydride, toluene, acetyl chloride and acetic acid.
- The pipe system used shall be able to withstand the corrosion with acetic anhydride, toluene, acetyl chloride and acetic acid.
- The valves shall be able to withstand the corrosion with acetic anhydride, toluene, acetyl chloride, and acetic acid.
- The sensors (Temperature, Pressure, and flow) shall withstand corrosion with acetic anhydride, toluene, acetyl chloride, and acetic acid.

21.4.4 Mechanical requirements

- 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 letting gas or vapor 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 sensors shall be made of stainless steel 316.
- The sensors shall be able to close the system completely.
- The sensors shall be able to read the data from the system.
- The Acetic Anhydride pilot plant shall be designed according to the *mechanical design*.

21.5 Pilot Plant test specification

21.5.1 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.

21.5.2 Prepare the reactor

1. Make sure all valves are closed
2. Make sure the power is turned off
3. Connect the reagent valve to the reactor
4. Reaction nb 1: Put the reagents amount needed in the reactor (amount of reagents: 4.88 kg Sodium bicarbonate, 74.4L vinegar)
5. Reaction nb 2: Put the new reagents amount needed in the reactor (amount of reagents: 3.77 L acetyl chloride, **5**L Toluene-Solvent)

6. Closed the valve for reagent filling.

21.5.3 Safety precaution

- The hot water (+100 °c) could suffer some burns (tank number 1 = Reactor)
- Toluene: Sweet aroma, hidden dangers - inhalation, skin, and fire risks; long-term impacts on organs, development, and nervous system.
- Acetyl chloride: corrosive, flammable, explosive, lung irritant, potential carcinogen.
- Acetic anhydride: highly corrosive, burns eyes and skin, inhaling damages lungs, potential carcinogen, flammable, and reacts violently with many substances.

(Wear protective gloves/protective clothing/eye protection/face protection).

21.5.4 Acetic Anhydride Production Operation

1. Ensure all sanitary connections
2. Put the dangerous reagents in the "reagents tanks" (tank A= Acetyl chloride and tank B= Acetone)
3. Put the reagents in the reactor (vinegar and sodium bicarbonate)
    1) Reaction 1: (vinegar or acetic acid 5%, sodium bicarbonate)
    2) Reaction 2: "solvent: toluene", sodium acetate and Acetyl chloride)
4. Plug the control system
5. Check the control system if it's working properly
6. Operate to boiling up the water (+100°c) in the "Jacket Reactor"
7. Operate the mixer to mix the reagents in the "Reactor"
8. Operate the reagents tanks (tank A and tank B) to transfer the reagents (from tank A and tank B to the "Reactor")
9. Operate to warm the water (50-80°c) in the "Jacket Reactor"
10. Operate to circling the water in the condenser
11. After finishing, operate the pipe to close.

21.5.5 001: ACETIC ANHYDRIDE PRODUCTION SYSTEM TEST

Step	**Step Description**	**Expected Result**
Precondition	System is OFF	
TURNING ON the system	Turn ON the GUI	The system is ON

Switch ON the heater (Jacket Reactor)	Turn ON the heater from the GUI	Reaction 1 THE HEATER is heating the water in the jacket till it reaches +100°C indicated on the TSJ (temperature sensor of the jacket)
Switch ON the mixer (Reactor tank)	Turn ON the mixer from the GUI	Mixing the reagents (manual added) to obtain the mixture in the "Reactor" till the water evaporated (creating anhydrous conditions)
Switch OFF the heater (Jacket Reactor)	Switch OFF the heater from the GUI	The water in the "Reactor jacket" and the mixture in the "Reactor" is cooled till it reaches 20-30°C, indicated on the TSJ (temperature sensor of the jacket)
1- Open the valve B (tank B: tank acetyl chloride) 2- Close the valve B (tank B: tank acetyl chloride)	1- Open the valve VB to transfer 3.77L acetyl chloride from Tank B to "Reactor" 2- Close the valve VB after transferring 3.77L acetyl chloride from tank B to "Reactor"	Reaction 2 1- 3.77L of Acetyl chloride is transferred to "Reactor" indicated on the FB (flow sensor tank B) 2- Valve B is closed after 3.77L is transferred to "Reactor" indicated on the FB (flow sensor tank B)
1- Open the valve A (tank A: tank Toluene) 2- Close the valve A (tank A: tank toluene)	1- Open the valve VA to transfer 5L toluene from Tank A to "Reactor" 2- Close the valve VB after transferred 5L toluene from tank A to "Reactor"	1- 5L of Toluene is transferred to "Reactor" indicated on the FA (flow sensor tank A) 2- Valve B is closed after 5L is transferred to "Reactor" indicated on the FA (flow sensor tank A)
Turn ON the water circulation in the condenser	Turn ON the water circulation in the condenser to start the distillation	The condenser is filled with water

Switch ON the heater (Jacket Reactor)	Turn ON the heater from the GUI	THE HEATER is heating the water in the jacket till it reaches 50-60°C indicated on the TSJ and TSR (temperature sensor of the jacket/Reactor)
After the reaction has completed Increase the Temperature of the heater (Jacket Reactor)	Increase the temperature of the heater from the **GUI**	Distillation (Toluene) The HEATER is heating the water in the jacket till it reaches 110°C indicated TSC (temperature sensor between the reactor and condenser)
1- Open the valve R-C (Reactor: Condenser) 2- Open the Valve P2 (tank P2: tank toluene)	1- Open the valve V R-C to transfer the vapor from Reactor to the condenser 2- Open the valve VP2 to transfer the liquid after distillation from the condenser to the tank P2	1- The vapor is transferred to "condenser" 2- The liquid is transferred to "Tank P2"
Increase the Temperature of the Heater (Jacket Reactor)	Increase the temperature of the Heater from the GUI	Distillation (Acetic Anhydride) THE HEATER is heating the water in the jacket till it reaches 139°C indicated TSC (temperature sensor between the reactor and condenser)
1. Open the valve R-C (Reactor: Condenser) 2. Open the Valve P1 (tank P1: tank acetic anhydride)	1. Open the valve V R-C to transfer the vapor from Reactor to condenser 2. Open the valve VP1 to transfer the liquid after distillation from condenser to tank P1	1. The vapor is transferred to "condenser" 2. The liquid is transferred to "Tank P1"
Switching OFF the system	Switch OFF the system	The system is OFF

Postcondition	System is OFF	

21.6 Mechanical Realization / Implementation Acetic Anhydride Pilot Plant

Condenser stainless-steel (inside view 1) 09.08.2024

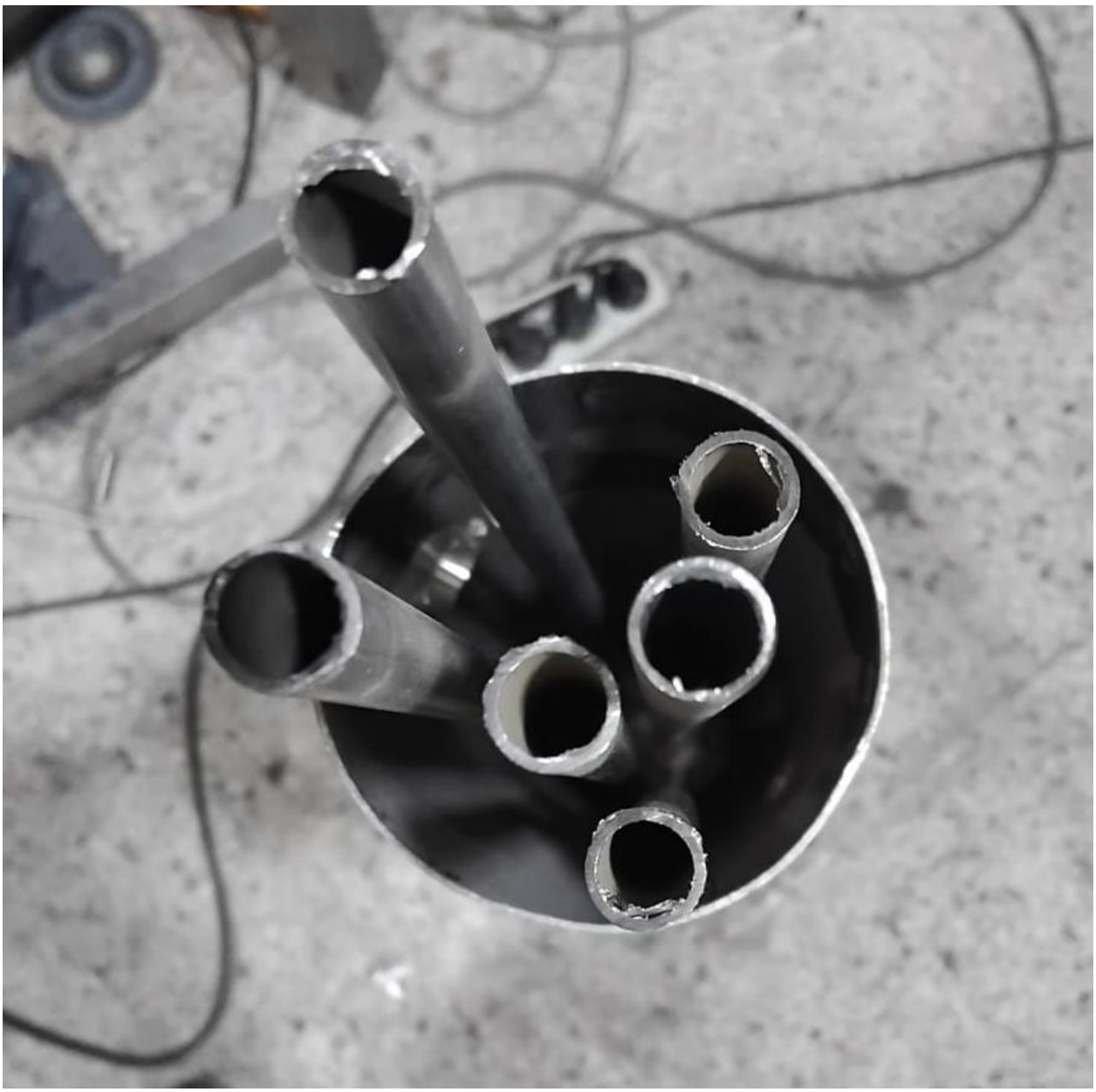
Condenser stainless-steel (inside view 2) 09.08.2024

Stainless-steel 316 female connector (condenser inlet) 14.08.2024

View 1

View 2

Outside condenser inlets and jacket 20.08.2024



Whole condenser inlets tubes and jacket (view 1) 10.09.2024

Whole condenser inlets tubes and jacket (view 2) 10.09.2024

Whole condenser inlets tubes and jacket (view 3) 10.09.2024

21.6.1 List of instruments and extensions for Acetic Anhydride Pilot Plant

- 2 Flow sensors (1/2 inches) – (stainless steel 316)
- 3 Temperature sensors (+100 °C) – (stainless steel 316)
- 5 electrical valves (1/2 inches) – (stainless steel 316)
- 2 Pressure sensors + 2 safety valves (1/2 inches) – (stainless steel 316)
- 1 mixer + axe (stainless steel 316)
- 4 manual valves stainless steel 316
- Heater

Price $???

21.7 Protocol Acetic Anhydride Production 16.02.2024

21.7.1 Introduction

"Acetic anhydride, a key ingredient in aspirin production, is a versatile chemical used in various industries." In this experiment, we will synthesize acetic anhydride from acetic acid and sodium bicarbonate.

21.7.2 Materials and Equipment

- Safety goggles
- Gloves
- Lab coat
- Reactor
- Condenser
- Temperature sensor x3
- Pressure sensor x2
- Flow sensor x2
- Heater
- Mixer
- Stainless steel tanks x4
- Vinegar (74.5L)
- Sodium bicarbonate (4.88kgs)
- Acetyl chloride (3.77L)

21.7.3 Procedure

1. In the "Reactor", Turn ON the mixer

   Add 74.5L vinegar and 4.88kgs sodium bicarbonate periodically to avoid fizzing reaction [Rx 1]

2. Turn ON the heating system in the jacket to reach 120 °C until the formation of sodium acetate to create anhydrous conditions
3. Cooldown between 50-80 °C, add 3.77L acetyl chloride and Turn ON the mixer [Rx 2]
4. Add 5L of toluene as "solvent" acts as a reaction medium and facilitator, enhancing interaction, controlling temperature, and aiding product isolation.
5. After the reaction completed, turn ON the water circulation in the condenser to start the distillation by turning ON the heating to 110 °C to evaporate the toluene (tank P1).
6. increase the temperature to 140 °C to evaporate the Acetic Anhydride (tank P2).
7. Turn OFF the system, test the purity and the yield of acetic anhydride

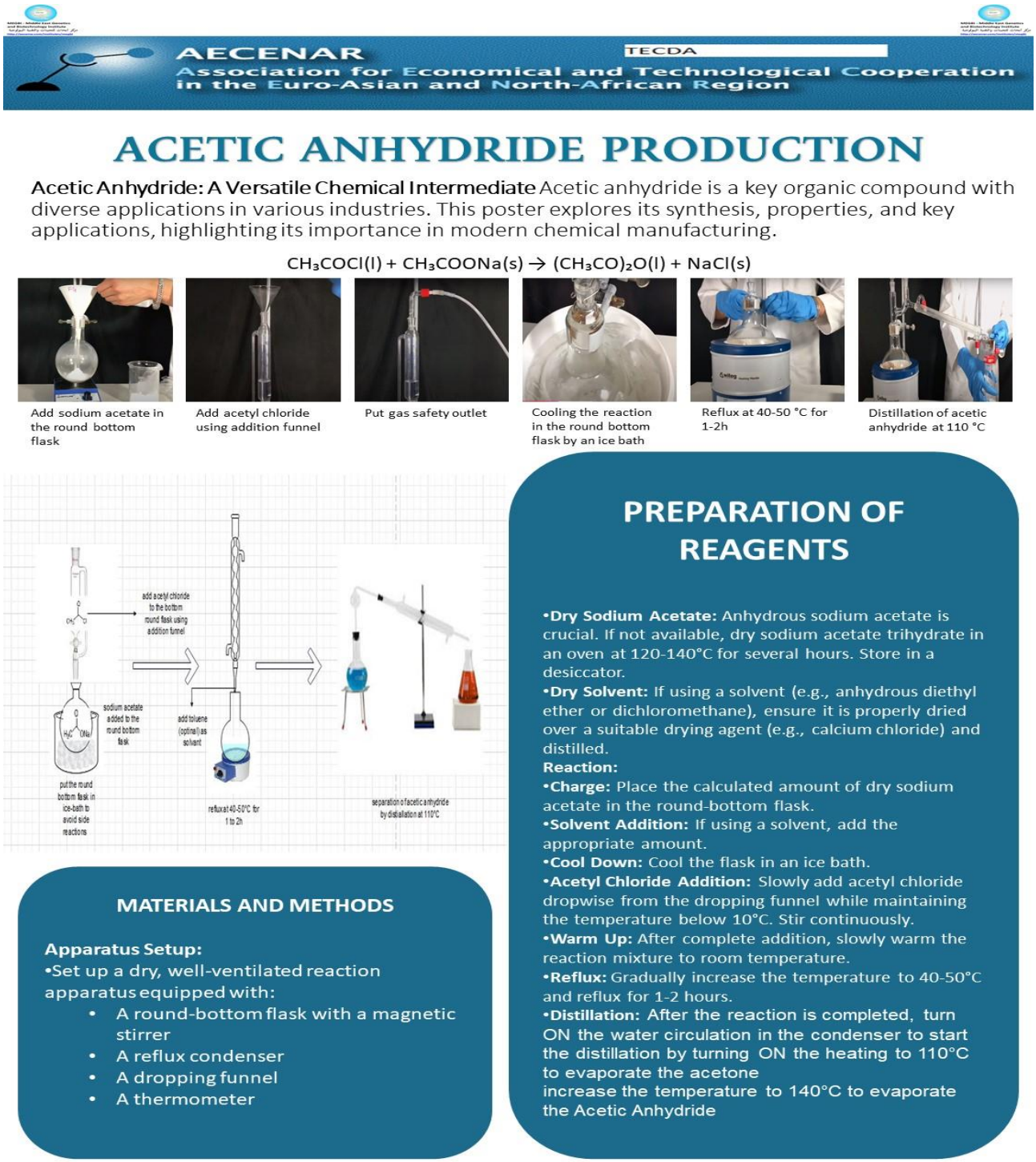
21.7.4 Safety

- -Toluene: Sweet aroma, hidden dangers - inhalation, skin, and fire risks; long-term impacts on organs, development, and nervous system.
- -Acetyl chloride: corrosive, flammable, explosive, lung irritant, potential carcinogen.
- -Acetic anhydride: highly corrosive, burns eyes and skin, inhaling damages lungs, potential carcinogen, flammable, and reacts violently with many substances.

⚠ N.B.: Always wear gloves and safety goggles when handling these chemicals

21.7.5 Result

21.8 Acetic anhydride Lab Scale Production



21.8.1 Synthesis of Acetic anhydride (PROTOCOL)

Aim: an experiment aims to provide a comprehensive understanding of both the theoretical and practical aspects of synthesizing acetic anhydride, which is a valuable intermediate in many industrial and laboratory processes.

- Material:

1. Vinegar (5%) or glacial acetic acid (CH_3COOH, 98%)
2. Sodium bicarbonate ($NaHCO_3$)
3. Acetyl chloride (C_2H_3ClO, 98%)
4. Toluene ($C_6H_5CH_3$) - optional

- Equipment:

1. Round-Bottom Flask
2. Beaker
3. Erlenmeyer flask
4. Digital balance
5. Spatula
6. Reflux Condenser
7. graduated cylinder
8. Aluminum
9. Hot plate
10. Distillation Apparatus
11. Droppers or Pipettes
12. Magnetic Stirrer
13. Thermometer
14. Ice Bath
15. Fume hood
16. Bunsen Burner
17. Protective Gear: Lab coat, gloves, and safety goggles for personal protection

- Reaction:

1. $NaHCO_3(s) + CH_3COOH\ (aq) \rightarrow CH_3COONa\ (s) + H_2O(l) + CO_2(g)$ "exothermic reaction"
2. $CH_3COONa(s) + CH_3COCl(aq) \rightarrow (CH_3CO)_2O(aq) + NaCl(s)$

- Procedure:

1. Setup the apparatus
2. Add 600ml of vinegar (5%) in a beaker under a Hot plate at T= 120°C and put a magnetic stirrer to mix the reaction
3. Weigh 42g using digital balance, then add gradually to the beaker to prevent excessive foaming
4. Start boiling to remove water, the liquid has crystals and becomes solid powder, which is sodium acetate, then the solution turns into a gooey paste
5. To fully dry the solid, melt it
6. Stop heating and the liquid was mixed by using a spatula that prevented it from solidifying
7. After mixing and cooling between 50-80°C, add 98.18 ml acetyl chloride
8. Add 1L of Toluene acts as a reaction medium and facilitator, enhancing interaction, controlling temperature, and aiding product isolation.
9. After the reaction is completed, turn ON the water circulation in the condenser to start the distillation by turning ON the heating to 110°C to evaporate the acetone
10. Increase the temperature to 140°C to evaporate the Acetic Anhydride
11. Turn OFF the system, test the purity and the yield of acetic anhydride.

21.8.2 HPLC Quantitative Analysis

HPLC: is a high-performance liquid chromatography that is widely used in analytical techniques and quantitative methods in the industry. Also, it provides accuracy and reliability.

Procedure:

1. We set up the equipment,
2. The mobile phase passes through a Gradient acetonitrile 'MeCN' – 20-100%, water, and phosphoric acid 15 min to degasser, that is, removing gas dissolve.
3. Then the mobile phase passes to the pump that maintains constant flow of mobile phase through the HPLC, following the sample injector, whereas the stationary phase is silica gel
4. Passing to column of Newcrom R1 is a special reverse-phase column with low silanol activity. It is based on spherical silica particles with 100 Å pores size and particle sizes of 3 μm and 5 μm. The stationary phase has advanced proprietary end-capping and is generally stable at basic pH values, with a recommended pH range of 1.0 to 10.0. Also, the principle of elution is dependent on the affinity, which is that the less polar will elute first and the more polar will elute second, whereas the flow rate is 5 ml/min
5. The elution will be entering the detector, 'Lambert Law," and will be LED light which passes to the filter, then to the sample and the absorbance value is detected at UV 200, 275nm
6. The light that passes through the sample will provide info of a particular wavelength that is converted to a digital signal and displayed in a monitor.
7. Monitor graph is absorbance versus retention time and shows chromatogram peaks.

21.8.3 INFRA-RED Method

IR: is infra-red, which is most useful in providing information about the presence or absence of specific functional groups. It also provides a molecular fingerprint that can be used when comparing samples. If two pure samples display the same IR spectrum, it can be argued that they are the same compound. Also, it is electromagnetic radiation

Preparation:

1. Set up the instrumentation by using a liquid cell with sodium chloride (NaCl) windows. Ensure the cell is clean and dry to prevent interference, followed by applying a thin film of the sample between the NaCl windows.

2. Then prepare a KBr pellet by grinding a small amount of KBr to a fine powder and mixing a small amount of your sample with the KBr powder, then pressing the mixture into a pellet using a hydraulic press.
3. IR radiation is generated by fitting a light source and directing it to the sample, "acetic anhydride synthesis "
4. Some light is reflected; the sample absorbs the specific amount of passing light.
5. The part of the light that is transmitted and carries the molecular information of the sample will be collected in acetic anhydride by a detector to produce electronic signals
6. To transfer the electronic signals into a spectrum, the light should first be directed to a diffraction grating, splitting into several beams traveling in a different direction
7. These beams were mechanically directed to the sample and each wavelength was examined individually
8. The Fourier Transform (FT) in FTIR spectroscopy is essential for converting the raw data collected from the sample (interferogram) into a usable IR spectrum. This process enables rapid, sensitive, and accurate analysis of the sample's molecular structure.
9. Analyze the IR spectrum. The two carbonyl groups in acid anhydrides give rise to two carbonyl stretching peaks. The vibrations involved are a symmetric C=O stretch where the two carbonyl groups stretch in phase with each other and an asymmetric C=O stretch where the two carbonyl groups stretch out of phase with each other

V = 1810-1750 cm^{-1}

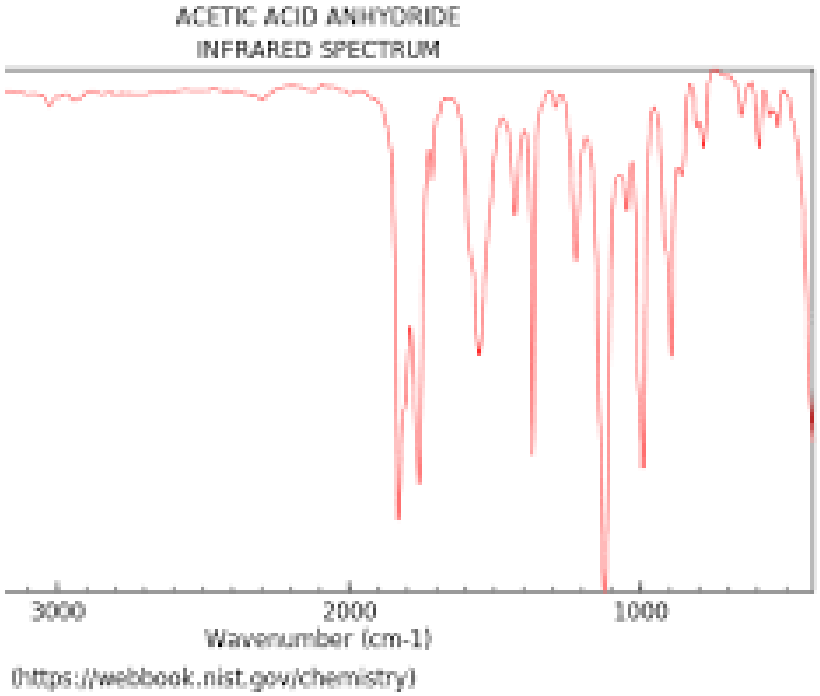
Additional Considerations

Solvent effects: If using a solvent, ensure it doesn't interfere with the target peaks.

Sample purity: Impurities can introduce additional peaks, complicating the spectrum interpretation.

Spectrum quality: Ensure a good signal-to-noise ratio for accurate peak identification.

By following these steps and carefully analyzing the IR spectrum, you should be able to confirm the presence of acetic anhydride in your sample.



22 Project 3: Sulfuryl Chloride Production Project

22.1 Objective of process control for Sulfuryl Chloride production

To produce sulfuryl chloride (SO_2Cl_2) by reacting sulfur dioxide (SO_2) with chlorine (Cl_2) in a controlled environment.

22.2 Materials and Equipment

22.2.1 Reactants

- Sulfur Dioxide (SO_2) - stored in pressurized tanks
- Chlorine (Cl_2) - stored in pressurized tanks

22.2.2 Catalyst (if applicable)

- Sodium fluoride-carbon catalyst (prepared as per the specifications)

22.2.3 Equipment

- 50-gallon stainless steel reaction vessel
- Stirring mechanism (mechanical stirrer)
- Cooling coils (for temperature control)
- Pressure gauge
- Off-gas line with a pressure relief valve
- Sampling apparatus
- Filtration system
- Storage containers for sulfuryl chloride

22.3 Safety Precautions

- Ensure all personnel are wearing appropriate personal protective equipment (PPE), including gloves, goggles, and lab coats.
- Work in a well-ventilated area or fume hood to avoid inhalation of gases.
- Have emergency equipment (eyewash station, safety shower, fire extinguisher).
- Be aware of the properties of chlorine and sulfur dioxide, as both are toxic and corrosive.

22.4 Preparation of Catalyst (if applicable)

Impregnation:

1. Boil activated carbon (e.g., Darco G-60) with a 4% aqueous solution of sodium fluoride.
2. Ensure that the sodium fluoride is thoroughly impregnated into the carbon particles.
3. Allow the catalyst to dry before use.

22.5 Setup of Reaction Vessel

22.5.1 Reactor Preparation

- Clean the stainless steel reaction vessel thoroughly.
- Install the stirring mechanism and cooling coils.

- Connect the off-gas line to a safe venting system.

22.5.2 Pressure and Temperature Control

- Ensure that the pressure gauge is calibrated and functioning.
- Set up the cooling system to maintain the reaction temperature below 55 °C.

22.6 Reaction Procedure

22.6.1 Cooling of Reactants

- Cool the chlorine and sulfur dioxide to their respective boiling points:
  - Chlorine: below -34.04 °C
  - Sulfur Dioxide: below -10 °C

22.6.2 Pressurization

- Pressurize the tanks containing chlorine to approximately 5-6 atmospheres.
- Pressurize the tanks containing sulfur dioxide to approximately 2-3 atmospheres.

22.6.3 Addition of Reactants

- Begin stirring the contents of the reaction vessel.
- Slowly add liquid chlorine and liquid sulfur dioxide in equimolar proportions (approximately 64 lbs. of SO_2 and 71 lbs. of Cl_2 per hour).
- Monitor the pressure and temperature continuously during the addition.

22.6.4 Monitoring the Reaction

- Analyze the reaction mixture periodically for unreacted gases using sampling apparatus.
- If unreacted chlorine is detected, add additional sulfur dioxide; if unreacted sulfur dioxide is detected, add additional chlorine.
- Maintain the temperature below 55 °C using the cooling coils.

22.6.5 Completion of Reaction

- After approximately 4-5 hours of continuous addition, stop the stirring.
- Allow the reaction mixture to settle for 1-2 hours.

22.7 Separation and Filtration

22.7.1 Withdrawal of Product

- Carefully withdraw the supernatant liquid (sulfuryl chloride) from the bottom of the reactor.
- Use a filtration system to remove any suspended solids or catalyst residues.

22.7.2 Storage of Sulfuryl Chloride

- Transfer the filtered sulfuryl chloride to appropriate storage containers.
- Ensure that the containers are sealed and labeled correctly.

22.8 Post-Reaction Cleanup

22.8.1 Cleaning the Reactor

- Cleaning the Reactor: Clean the reaction vessel and all equipment used in the process to remove any residual chemicals.
- Dispose of any waste materials according to local regulations.

22.8.2 Safety Check

- Conduct a safety check of the area to ensure no leaks or residual gases are present.

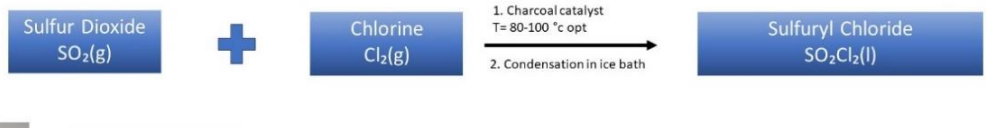
22.9 Sulfuryl Chloride Lab Scale production

بسم الله الرحمن الرحيم

AECENAR
TECDA
Association for Economical and Technological Cooperation in the Euro-Asian and North-African Region

Sulfuryl Chloride Production

Introduction: **Acetyl sulfuryl chloride (CH_3COSO_2Cl)** is a valuable reagent in organic synthesis, offering unique reactivity profiles for various transformations. This poster will explore the synthesis of acetyl sulfuryl chloride, highlighting its significance and potential applications in chemical research.




2x Gaz cylinders for $SO_2(g)$ And $Cl_2(g)$


Pressure gauge


Magnetic stirrer

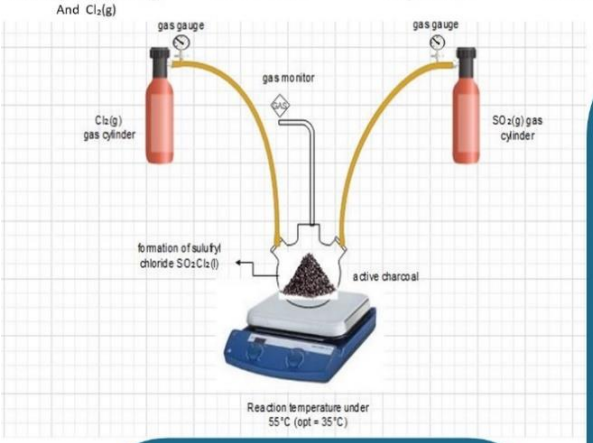

condenser


PPE safety


spectrophotometer


Gas chromatography


Round bottom flask multi neck



MATERIALS AND METHODS

Materials:

Materials and Methods

1.Catalyst Preparation:

1. Use activated carbon (e.g., Darco G-60).
2. Impregnate with a solution of sodium fluoride (NaF) or other metal salts.
3. Example preparation: Boil a 4% sodium fluoride solution with carbon for 1-2 hours, then filter and dry.

2.Experimental Setup:

1. Reactor setup for liquid phase or vapor phase reactions.
2. Ensure proper handling of toxic gases (SO_2 and Cl_2) in a fume hood.

TESTING

1. Gas Chromatography (GC)

•**Purpose**: To analyze the composition of the gas phase and detect unreacted sulfur dioxide (SO_2) and chlorine (Cl_2).

3. Infrared Spectroscopy (IR)

•**Purpose**: To identify and quantify the presence of sulfuryl chloride and other reactants/products in the liquid phase.

REACTION CONDITIONS

1.Reactor Setup:

1. **Type**: 1-liter round-bottom flask or small stainless-steel reactor.
2. **Stirring**: Magnetic stirrer with a stir bar to ensure thorough mixing of reactants.
3. **Cooling**: Cooling bath (ice or water) to maintain the reaction temperature.

2.Temperature:

1. **Optimal Range**: Maintain the temperature of the reaction mass below 55 °C.
2. **Preferred Range**: Ideally, operate within 30-40 °C for better control and efficiency.

3.Pressure:

1. **Initial Pressure**: Atmospheric pressure (1 atm) is typically sufficient.
2. **Maximum Pressure**: Monitor and prevent pressure buildup; vent excess gases if necessary.
3. **Operating Pressure**: Can be adjusted up to 25 lbs. or higher if needed, but this is not typically required for laboratory-scale reactions.

4.Reactant Proportions:

1. **Equimolar Ratio**: Introduce sulfur dioxide (SO_2) and chlorine (Cl_2) in equimolar proportions.
2. **Example Quantities**: For a small-scale reaction, approximately 10 mL of each gas can be used.

5.Catalyst Preparation:

1. **Type**: Sodium fluoride-activated carbon catalyst.
2. **Amount**: Approximately 5 grams of the prepared catalyst.
3. **Preparation Method**: Impregnate activated carbon (e.g., Darco G-60) with a 4% aqueous solution of sodium fluoride, then dry.

6.Reaction Time:

1. **Duration**: Continuous addition of SO_2 and Cl_2 for about 1-2 hours, depending on the desired yield.
2. **Monitoring**: Regularly check for unreacted gases and adjust the addition rate accordingly.

7.Gas Flow Rates:

1. **Controlled Addition**: Use gas flow meters to control the rate of SO_2 and Cl_2 addition.
2. **Example Flow Rates**: Approximately 64 lbs. of SO_2 and 71 lbs. of Cl_2 per hour for larger setups; adjust for smaller laboratory-scale reactions.

8.Post-Reaction Conditions:

1. **Settling Time**: Allow the reaction mass to settle for 30 minutes to 1 hour after the reaction is complete.
2. **Filtration**: Filter the supernatant liquid to remove any suspended catalyst particles.

Mohammad Kalawoun@ AECENAR_Green chemistry /December 2024

Poster of Sulfuryl Chloride Production [pptx file]:	sulfuryl chloride production.pptx

22.9.1 Reaction Equation

$Cl_2(g) + SO_2(g) \rightarrow SO_2Cl_2(l)$

22.9.2 Laboratory Setup:

1. **Gas Cylinders:** Obtain gas cylinders of chlorine and sulfur dioxide. Ensure that the cylinders are equipped with pressure regulators and flow meters.
2. **Glassware:**
    - **Reaction Flask:** A round-bottom flask with a capacity of 250-500 mL is suitable.
    - **Condenser:** A reflux condenser to condense the sulfuryl chloride vapor.
    - **Drying Tube:** A drying tube filled with anhydrous calcium chloride or concentrated sulfuric acid to remove any moisture from the gases.
    - **Gas Delivery Tubes:** Glass tubes with rubber tubing connections to connect the gas cylinders to the reaction flask.
3. **Catalyst:** Prepare a charcoal catalyst with 4% fluorine. This can be done by impregnating activated charcoal with a fluorine-containing compound, such as ammonium fluoride or hydrofluoric acid.
4. **Heating Source:** A hot plate or heating mantle to heat the reaction flask.
5. **Ice Bath:** A beaker filled with ice to cool the condenser and collect the sulfuryl chloride.

22.9.3 Experimental Procedure:

1. **Set Up the Apparatus:**
    - Connect the gas cylinders to the reaction flask using the gas delivery tubes.
    - Insert the condenser into the reaction flask, and connect the drying tube to the condenser.
    - Place the ice bath around the condenser.
2. **Add the Catalyst:**
    - Weigh the desired amount of the prepared charcoal catalyst and add it to the reaction flask.
3. **Introduce the Gases:**
    - Slowly open the valves on the gas cylinders to allow a steady flow of chlorine and sulfur dioxide into the reaction flask.
    - The flow rates can be adjusted using the flow meters.
4. **Heat the Reaction:**

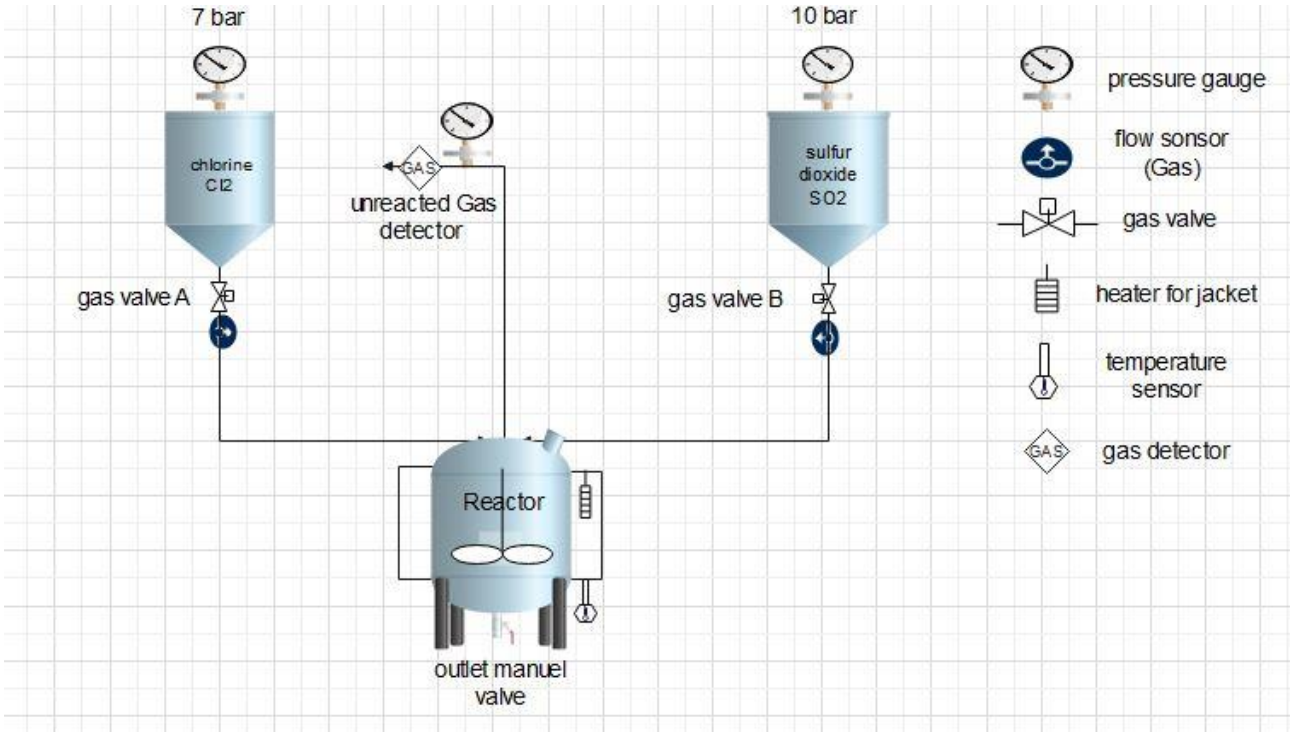
- Turn on the heating source to heat the reaction flask to a temperature of 80-100°C.
- The reaction will proceed at this temperature.

5. **Collect the Product:**
   - The sulfuryl chloride product will condense in the condenser and collect in the ice bath.
6. **Monitor the Reaction:**
   - Periodically check the flow rate of the gases and the temperature of the reaction.
   - Once the reaction is complete, the flow of gases can be stopped.
7. **Isolate the Product:**
   - Remove the condenser from the ice bath and carefully transfer the sulfuryl chloride to a suitable container.
   - The product can be further purified by distillation, if necessary.

22.9.4 Safety Precautions:

- Chlorine and sulfur dioxide are toxic gases. Work in a well-ventilated area and wear appropriate respiratory protection.
- Hydrofluoric acid is a highly corrosive and dangerous chemical. Handle it with extreme caution and wear protective gloves and eye protection.
- The reaction should be carried out in a fume hood to prevent the release of toxic gases.

22.10 Sulfuryl chloride Pilot Scale production



Flow chart of sulfuryl chloride [Edraw file]:

map sulfuryl chloride.eddx

22.11 Compressor for Cl_2 and SO_2

Q1: Can we use a normal air compressor to compress $Cl_2(g)$ and SO_2 into the gas cylinders?

R1:

Chlorine gas Cl2:

No, you should **not** use a normal air compressor to compress chlorine gas (Cl2) into a gas cylinder. Here's why:

1. Compatibility: Normal air compressors are designed for air, which is a mixture of primarily nitrogen and oxygen. Chlorine gas is a highly reactive and corrosive substance that can damage the internal components of a standard air compressor.

2. Safety Hazards: Chlorine gas is a toxic and potentially lethal substance. If a **leak** occurs during compression, it can pose a **serious health risk to anyone** in the vicinity. Standard air compressors are not designed to handle hazardous gases and may not have the necessary safety features to prevent leaks or contain a release.

3. Equipment Requirements: Compressing chlorine gas requires specialized equipment designed to handle its corrosive properties and ensure safe operation. This includes:

    - Chlorine-specific compressor: These compressors are made from materials resistant to chlorine, such as stainless steel or special alloys.
    - Safety features: These may include leak detection systems, emergency shutoff valves, and containment measures to minimize the risk of exposure in case of a leak.
    - Proper ventilation: Adequate ventilation is crucial to prevent the buildup of chlorine gas, which can be dangerous at high concentrations.

If you need to compress chlorine gas, it is essential to consult with experts in gas handling and safety. They can advise you on the appropriate equipment, procedures, and safety precautions.

Sulfur dioxide Gas SO2:

No, you should not use a normal air compressor to compress sulfur dioxide (SO2) gas into a gas cylinder. Here's why:

1. Compatibility: Normal air compressors are designed for air, which is a mixture of primarily nitrogen and oxygen. Sulfur dioxide is a corrosive gas that can damage the internal components of a standard air compressor.

2. Safety Hazards: Sulfur dioxide is a toxic gas that can irritate the respiratory system. If a leak occurs during compression, it can pose a serious health risk to anyone in the vicinity.

Standard air compressors are not designed to handle hazardous gases and may not have the necessary safety features to prevent leaks or contain a release.

3. Equipment Requirements: Compressing sulfur dioxide gas requires specialized equipment designed to handle its corrosive properties and ensure safe operation. This includes:

    - SO2-specific compressor: These compressors are made from materials resistant to sulfur dioxide, such as stainless steel or special alloys.
    - Safety features: These may include leak detection systems, emergency shutoff valves, and containment measures to minimize the risk of exposure in case of a leak.
    - Proper ventilation: Adequate ventilation is crucial to prevent the buildup of sulfur dioxide gas, which can be dangerous at high concentrations.

Solution:

For compressing chlorine (Cl2) and sulfur dioxide (SO2) gases, you should use specialized compressors designed to handle these corrosive and hazardous substances.

Here are some suitable options:

- Diaphragm compressors: These compressors use a flexible diaphragm to separate the gas from the compressor's internal components, minimizing the risk of corrosion and contamination.
- Liquid ring compressors: These compressors use a rotating impeller to displace the gas, with a liquid seal to prevent leakage and corrosion.
- Screw compressors: Some specially designed screw compressors with corrosion-resistant materials and coatings can be used for certain applications involving these gases.

Important Considerations:

- Materials of Construction: The compressor and its components must be made from materials resistant to the specific gas (e.g., stainless steel, Hastelloy, Teflon).
- Safety Features: The compressor should incorporate safety features such as leak detection systems, emergency shutoff valves, and pressure relief devices.
- Ventilation: Adequate ventilation is crucial to prevent the buildup of hazardous gases in the work area.

23 Project 5: Sulfur dioxide Production Project

23.1 Sulfur dioxide Lab Scale Production

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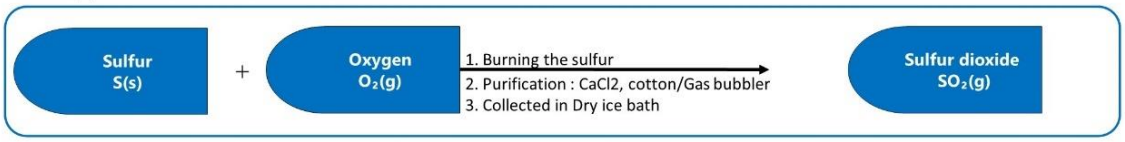
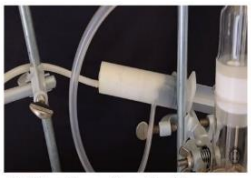
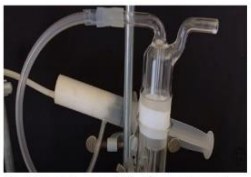
Sulfur Dioxide Production

Introduction: Sulfur dioxide (SO_2) is an important industrial chemical with various applications. One of the most common methods for producing SO_2 is by directly burning elemental sulfur in the presence of oxygen.
This reaction readily occurs at elevated temperatures, yielding sulfur dioxide gas as the primary product.
This poster will explore the key aspects of this production method, including the reaction mechanism, process conditions, and industrial applications of sulfur dioxide.



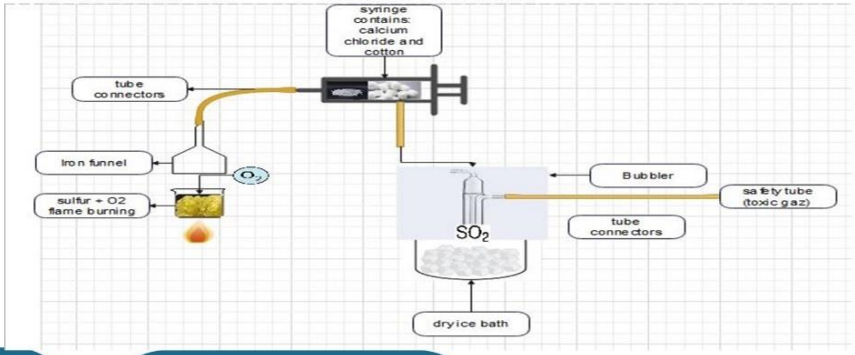

1. Burning the Sulfur powder in presence of O_2 with fire gun.


2. Filter the sulfur dioxide through a syringe contain cotton and calcium chloride $CaCl_2$.


3. After the purification step the gas should go through the bubbler connected to collection tube.


4. Put the collection tube in dry ice bath.


5. Sulfur dioxide is transformed to liquid form



MATERIALS AND METHODS

Materials:

- Elemental sulfur (powdered or chunks)
- Oxygen gas source
- Heat source (Bunsen burner or fire gun)
- Glassware (bubbler gas, iron funnel, collection tubes, delivery tubes, dry ice bath, Large Syringe, tyau caoutchouc)

CONDITIONS

Conditions Needed for Sulfur Combustion to Produce SO2
To ensure the efficient combustion of sulfur and the production of sulfur dioxide (SO2), the following conditions are essential:

1.Sufficient Oxygen Supply:
- •A plentiful supply of oxygen is crucial for the reaction to proceed.
- •Air, which contains approximately 21% oxygen, can be used as the oxygen source.

2.Ignition Source:
- •A heat source, such as a flame or a spark, is required to initiate the combustion process.

3.Adequate Temperature:
- •The reaction is exothermic, meaning it releases heat. However, an initial input of heat energy is necessary to start the reaction.
- •Once initiated, the reaction sustains itself due to the heat released.

TESTING

1.Water Test:
- •Bubble the gas through water.
- •SO2 reacts with water to form sulfurous acid, which is acidic.
- •Dip blue litmus paper into the solution. A color change to red confirms the presence of an acid, indicating the presence of SO2.

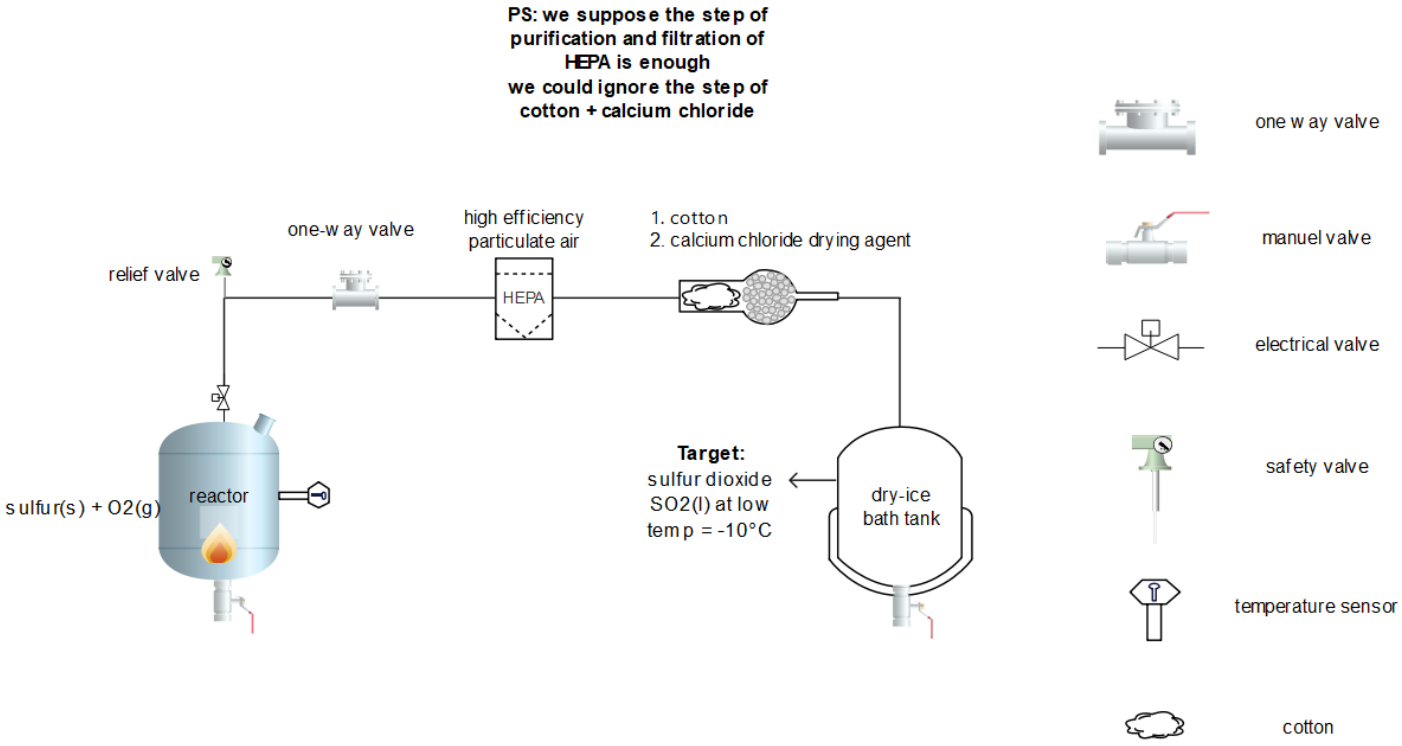
2.Potassium Permanganate Test:
- •Bubble the gas through a potassium permanganate solution.
- •SO2 is a reducing agent and will decolorize the purple potassium permanganate solution.

3.Iodine-Starch Paper Test:
- •Moisten a piece of starch-iodine paper with water.
- •Expose the moistened paper to the gas.
- •SO2 will react with iodine, causing the paper to lose its blue-black color.

Mohammad kalawoun @Green Chemistry/AECENAR 2024/2025

Poster of Sulfur Dioxide Lab Scale production [pptx file]:	sullfur dioxide production poster.p

23.2 Sulfur dioxide pilot plant scale production



Flow chart of Sulfur Dioxide [Edraw file]:

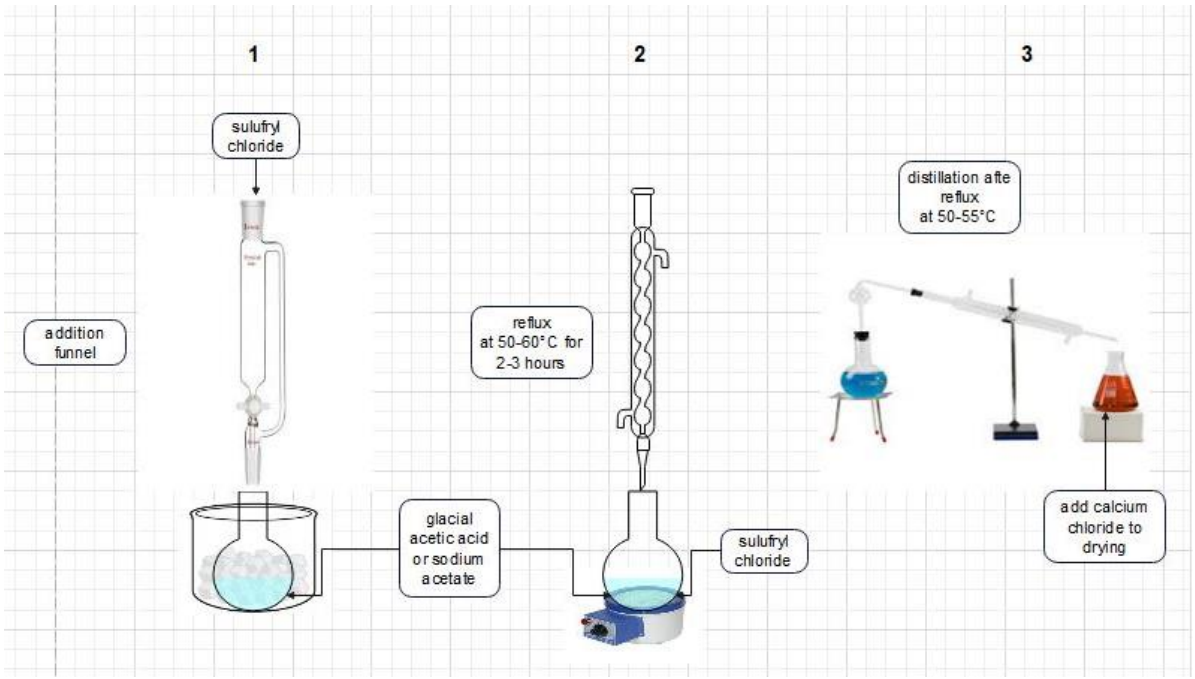
map sulfur dioxide.eddx

24 The two pathways for Acetyl Chloride Production

24.1 Acetyl Chloride Production with sulfuryl chloride (sulfur) as basic matrial (Reaction 2a and 2b)

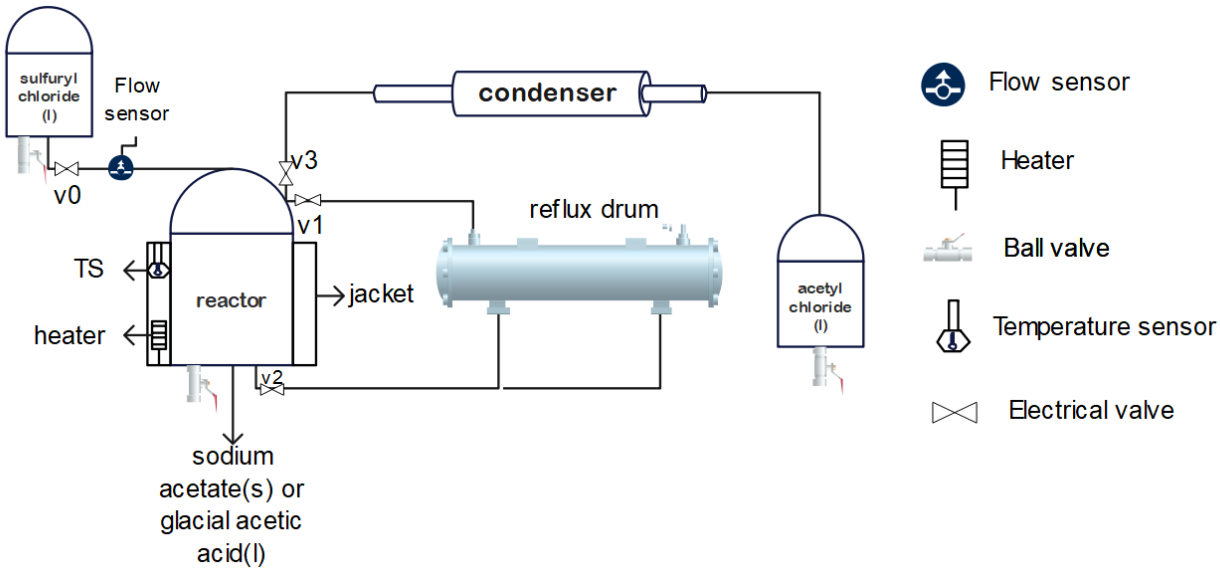
24.1.1 Lab scale reaction (2a)

23.12.2024: Flow chart production of acetyl chloride



map acetyl chloride.eddx

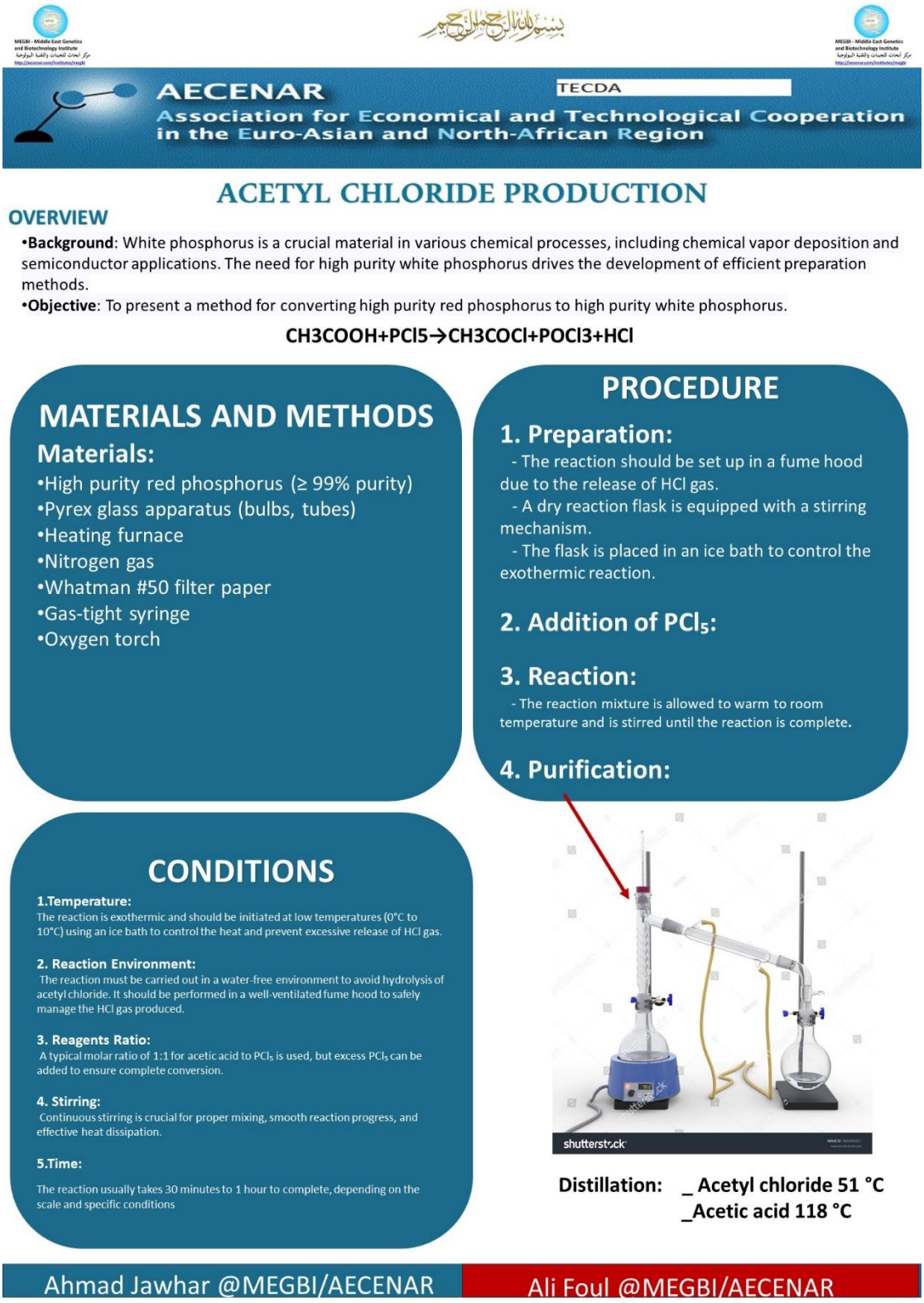
24.1.2 Pilot Plant scale reaction (2a)



Flow Chart of Acetyl Chloride [Edraw file]:

24.2 Acetyl Chloride Lab Scale Production with Phosphor as basic material

24.2.1 Phosphor based Acetyl chloride Lab scale Production



Poster of Acetyl chloride [pptx file]:	W.P. Poster.pptx

The two pathways for Acetyl Chloride Production

24.2.2 Phosphor based Acetyl chloride Pilot Plant Scale Production

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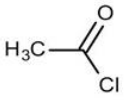
Acetyl chloride production scale

Introduction: Acetyl chloride (CH_3COCl) is an acyl chloride used in various organic synthesis reactions, particularly for introducing acetyl groups. One common method of synthesis is by reacting acetic acid with phosphorus pentachloride (PCl_5), a strong chlorinating agent.

Alternate Name:
1.Acetic acid chloride
2.Ethanoyl Chloride
3.Acyl Chloride



Chemical structure

Objective: This poster explores the chemical synthesis process of acetyl chloride and the reaction conditions necessary for optimal production

Chemical reaction:

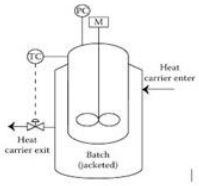
$$CH_3COOH + PCl_5 \rightarrow CH_3COCl + POCl_3 + HCl$$

In this reaction, phosphorus pentachloride replaces the hydroxyl group (-OH) in acetic acid with a chlorine atom, forming acetyl chloride. The by-products are phosphorus oxychloride ($POCl_3$) and hydrogen chloride (HCl)

Parameter	condition	Explanation
Time	1to 2 hours	
Reaction type	Exothermic	Heat is released during the reaction, which needs to be controlled.
Temperature	20–40°C (room temperature to slightly elevated)	Optimal for the reaction, prevents decomposition of acetyl chloride.
Pressure	Optimal for the reaction, prevents decomposition of acetyl chloride.	No pressure requirements

Unit operation needed:

Reactor : Since this is a relatively simple, exothermic reaction, a batch reactor may be appropriate for small-scale synthesis, allowing for careful control of reactant addition



3_phase separator :
3-phase separator in acetyl chloride synthesis separates hydrogen chloride gas (HCl), acetyl chloride (light liquid), and phosphorus oxychloride (heavy liquid). HCl gas is vented to a scrubber, while acetyl chloride and phosphorus oxychloride are separated based on density. This ensures efficient separation and safe handling of corrosive by-products.

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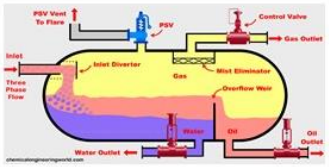
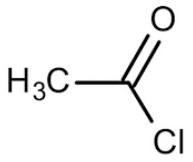


Poster of *Acetyl chloride production scale* [pptx file]:

10-09-24Acetyl chloride production

The two pathways for Acetyl Chloride Production

24.2.2.1 Chemical structure of Acetyl chloride:



24.2.2.2 Chemical and physical properties of Acetyl chloride

Molecular formula	CH3COCl
Density	1.1 g/cm³
Molar mass	78.94 g/mol
Boiling point	51℃
flash point	4 ℃

24.2.2.3 Alternative Names of Acetyl chloride

- Acetic acid chloride
- Ethanoyl Chloride
- Acyl Chloride

24.2.2.4 First reaction and typically used in the formation at AECENAR

CH3COOH +PCl5 → CH3COCl +PCl3 +HCl

24.2.2.5 Second reaction of Acetyl chloride

3CH3COOH+PCl5→ CH3COCl +POCl3 +HCl (exothermic reaction)

Acetyl chloride is toxic and corrosive.

24.2.2.6 Storage of Acetyl chloride

- Store in dry, well-ventilated area
- Take all necessary precautions to avoid the accidental
- keep container tightly closed
- Shelf life is 24 months

24.2.2.7 Unit operation needed :

Acetyl chloride needs

1. Reactor (Batch or CSTR), Batch reactor is better in this case
2. Distillation column or phase separator

The table shows the difference in the type of reactor and the price:

The two pathways for Acetyl Chloride Production

3000L jacketed	10800$
800L	7200$
50L emulsifying	3200$

Determining the exact price:

- Reactor size Volume
- Materials of construction
- operating condition
- Design specification

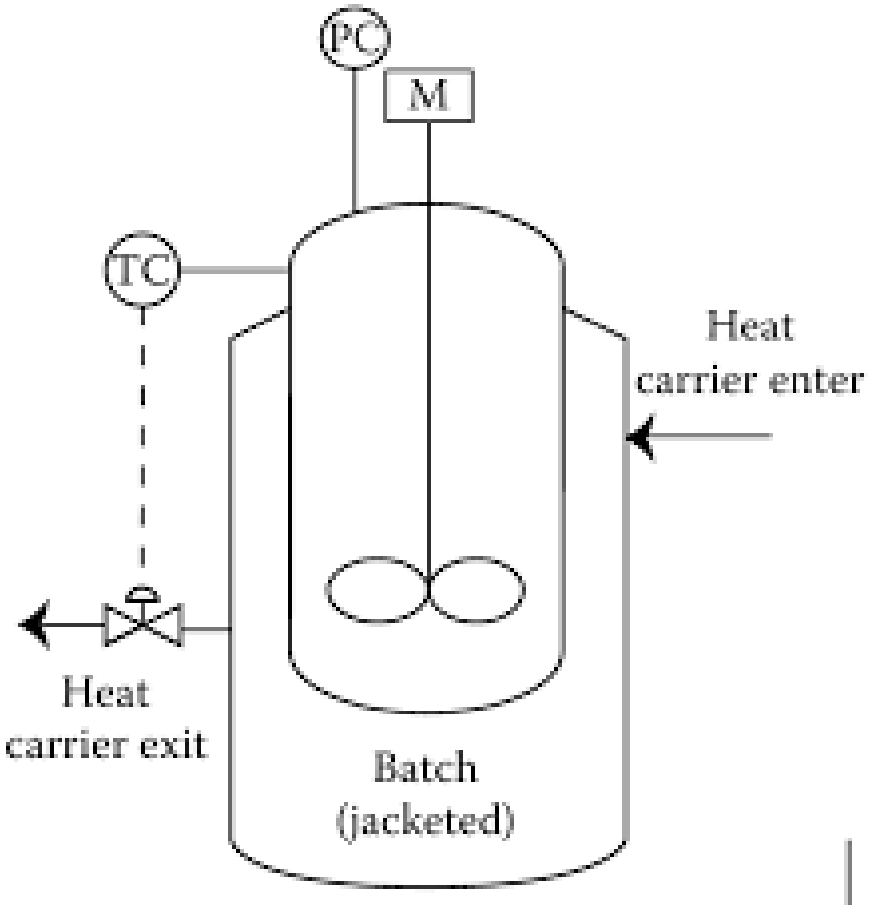
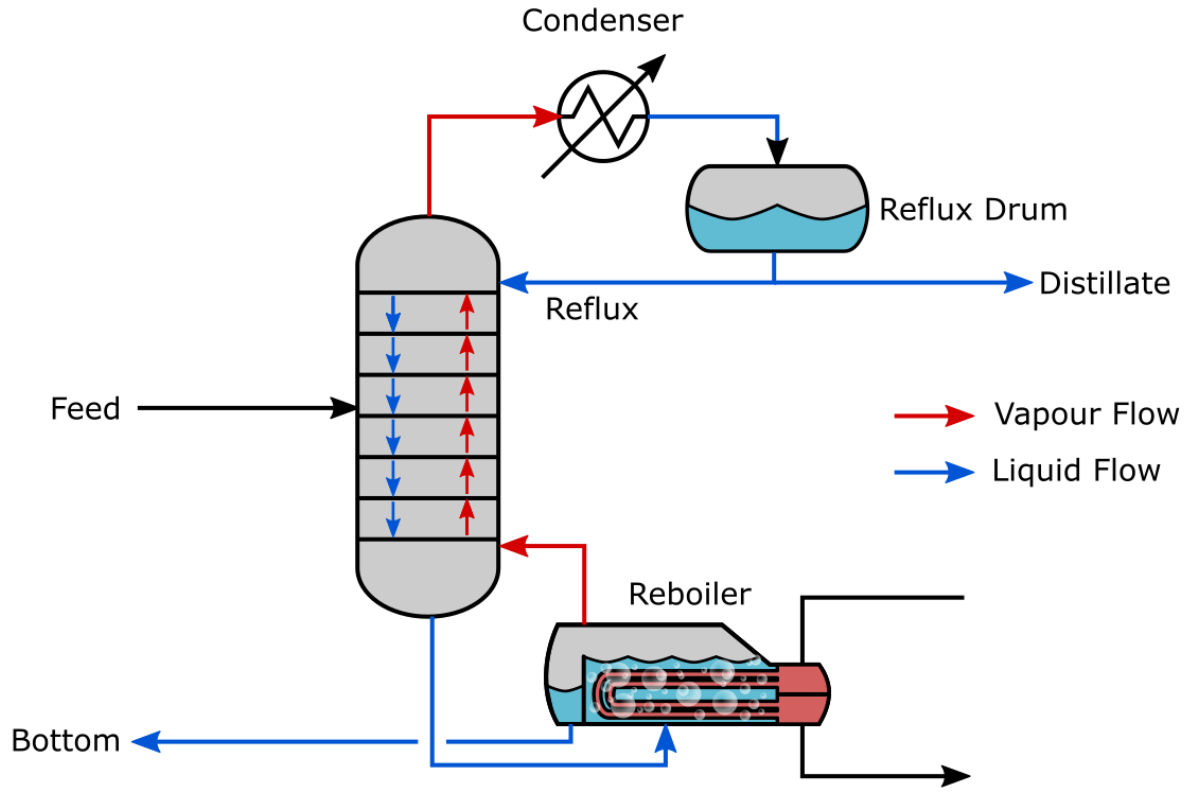


Figure 2: Batch jacketed reactor

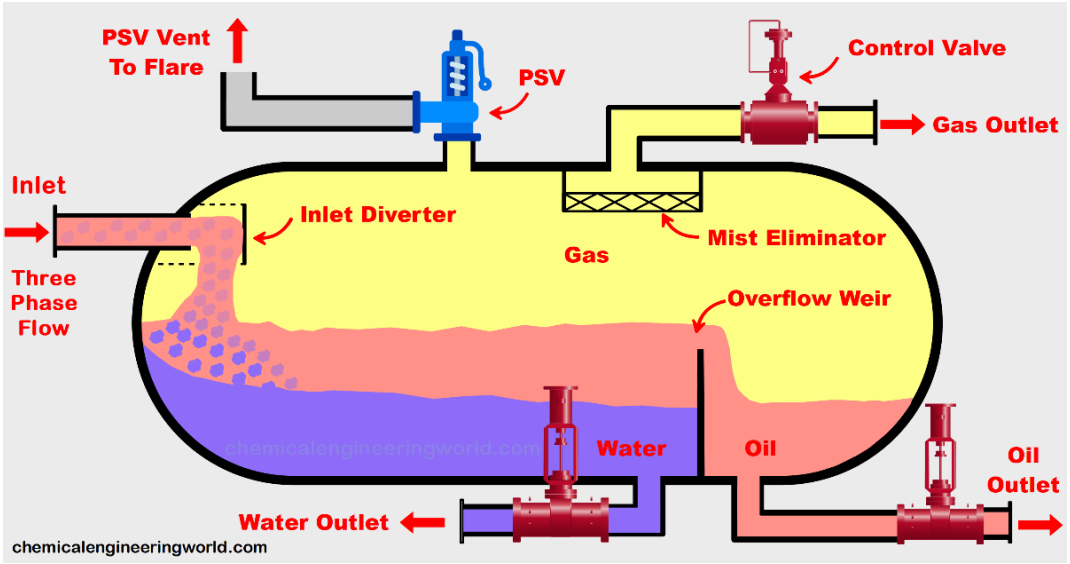
Distillation column or phase separator:

- Use a Distillation Column: A distillation column would be the better choice if your goal is to obtain high-purity acetyl chloride, especially in a continuous production process.

The two pathways for Acetyl Chloride Production



- Use a Phase Separator: If the mixture has clear immiscible phases and purity is less critical, or if you are looking for a more energy-efficient and simpler process, a phase separator might suffice.



24.2.2.8 Unit operation needed in the chemical industry

Momentum transport operation: pumps, pipes, compressor.

Heat transfer operation: change temperature

Mass transfer operation: distillation, absorption, extraction.

Chemical reaction operation: batch reactor, tubular.

Mechanical operation

References

https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&cad=rja&uact=8&ved=2ahUKEwi0ssvO86iIAxVJAvsDHcI4Lf8QFnoECBQQAw&url=https%3A%2F%2Fwww.sciencedirect.com%2Ftopics%2Fchemistry%2Ftablet-coating%23%3A~%3Atext%3DTablet%2520coating%2520is%2520the%2520process%2520where%2520coating%2520material%2520is%2520applied%2Cand%2520color%2520of%2520the%2520drug&usg=AOvVaw3i_tbYpeGn_jsifDP6qFzv&opi=89978449

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