بسم الله الرحمن الرحيم

MEGBI Antibiotics Production Pilot Plant (MEGBI-APP)

- 6th Project Report (Apr 2018 - Feb 2019) -

- تصنيع بنتسيلين (penicillin) على مستوى مختبر (laboratory scale penicillin production)
- Completing integration of MEGBI-APP test rig (in-built steam autoclaving unit)

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المضمون

1 Introduction

1.1 Production of penicilline

Penicillium cells are cultured using a Fed-batch culture technique. The fermentation of the mushrooms is done from cultures in depths in liquid mediums located in tanks where they multiply.

A study shows that when the temperature equal to 22 (+2) °C and the pH was kept at 6 and when the concentration of lactose is 60 kg / m^3 and that of cornsteep is 30 kg/m^3 in the medium the maximum yield of production penicillin for the substrate was available.[1]

In another study the researchers obtain an optimal culture medium for the growth of *Penicillium chrysogenum*, a medium was prepared with 3 g of yeast extract and 21 g of sucrose in one liter of pure distilled water. Temperature is a factor influencing the synthesis The highest production rate was at 28 ° C. The rate of penicillin production was significantly reduced at temperatures above 30°C (Asnaashari, Ghanbary, & Tazick, 2012).

In a study conducted by *Penicillium chrysogenum* spore survival in the light of UV rays and their production of antibiotics, was studied. The results obtained showed that changes in *P. chrysogenum* due to mutation, leading to more penicillin production. In one survey, antibacterial and antifungal compounds obtained from fungal species isolated from agricultural lands in northern Iran were studied (Gharaei F et al, 2009).

1.2 time plan for production of penicillin in Ras Maska Lab

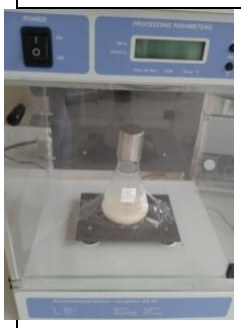
Date	Lab
19-04-2018	Culture of fungi
21-04-2018	preparation of liquid medium culture after 7 days
27-04- 2018	Continue of Protocol experiment • Filtration • Incubation of charcoal treatment • incubation with ethyl acetate

[1] Production of penicillin by fermentation 2003-2018 www.healtheappiontments.com

28 -04-2018	• Centrifugation • Addition of sodium bicarbonate • Incubation in refrigerator
several weeks	• long time incubation in the refrigerant after filtration obtaining peniccillin crystals • Production hypophilization obtaining penicillin poudre

2 طرُق العمل (methods and materials) لإنتاج البنسلين

2.1 الاجهزة اللازمة (Devices) للانتاج على مستور المختبر (lab scale production)

Incubator	centrifuge	magnetic agitator	refrigerator	microscope
				

2.2 المواد اللازمة (Materials) للانتاج على مستور المختبر (lab scale production)

	Peptone (Erlenmeyer de 100ml of volume de peptone), glucose, lactose, KCl, $MgCl_2$, KH_2PO_4, distilled water, Ethyl acetate
	Acetic Acid, Sulfuric acide, Spririt of vinegar ethanol

2.3 List of materiel purety from sigma and www.alibaba.com

SIGMA %PURETY MATERIELS

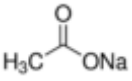
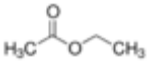
Pentyl acetate	lactosev	Sodium phosphate dibasic
227471	61345	• 14 Product Results • | Match Criteria: Formula
Amyl acetate, mixture of isomers	D-Lactose monohydrate	Na_2HPO_4
Mixture of 2-methylbutyl acetate and *n*-pentyl acetate, 99%	≥98.0% (HPLC)	• Synonym: *sec*-Sodium phosphate, Disodium hydrogen phosphate, Disodium phosphate, Sodium hydrogenphosphate • ○ Linear Formula: Na_2HPO_4 ○ Molecular Weight: 141.96 ○ CAS Number: 7558-79-4
109584	61339	• 
Pentyl acetate	D-Lactose monohydrate	• S7907
99%	BioUltra, ≥99.5% (HPLC)	• BioXtra, ≥99.0% (Sigma-Aldrich)
46022	L3625	•
Pentyl acetate	α-Lactose monohydrate	• pricing
puriss. p.a., ≥98.5% (GC)	≥99% total lactose basis (GC)	• SDS
8.18700	L8783	• 
n-Amyl acetate	α-Lactose monohydrate	• 795410
EMPLURA®	BioXtra, ≥99% total lactose basis (GC)	• anhydrous, free-flowing, Redi-Dri™, ACS reagent, ≥99% (Sigma-Aldrich)
66962	M9171	•
Pentyl acetate	D-(+)-Maltose monohydrate	• pricing
analytical standard	BioXtra, ≥99%	• SDS
174092		• 
2-Pentyl acetate		• S3264
99%		• for molecular biology, ≥98.5% (titration) (Sigma)
W205508		•
Isoamyl acetate		• pricing
≥95%, FCC		• SDS
		• S5136
W205532		• BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99.0% (Sigma)
Isoamyl acetate		• pricing • SDS

natural, ≥97%, FCC, FG 398268 1-Pentanol ACS reagent, ≥99% 09817 Collodion solution for microscopy, 2% in amyl acetate		•  • 255793 • 99.95% trace metals basis (Aldrich) • pricing • SDS

$MgSO_4 \cdot 7H_2O$ 63138 Magnesium sulfate heptahydrate BioUltra, ≥99.5% (KT) C0626 N-Z-Amine® A from bovine milk, Casein enzymatic hydrolysate 230391 Magnesium sulfate heptahydrate ACS reagent, ≥98%	$FeSO_4 \cdot 7H_2O$ 1270355 Ferrous sulfate United States Pharmacopeia (USP) Reference Standard $FeSO_4 \cdot 7H_2O$ F7002 Iron(II) sulfate heptahydrate *ReagentPlus*®, ≥99% $FeSO_4 \cdot 7H_2O$ F8633 Iron(II) sulfate heptahydrate BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99%	K_2HPO_4 P8281 Potassium phosphate dibasic reagent grade, ≥98.0%

• Sucrose • S7903 • BioXtra, ≥99.5% (GC) (Sigma) •  • pricing • SDS • • 84097 • BioUltra, for molecular biology, ≥99.5% (HPLC)	$ZnSO_4 \cdot 7H_2O$ Z0251 Zinc sulfate heptahydrate BioReagent, suitable for cell culture	$(NH_4)_2SO_4$ 221252 Ammonium sulfate ACS reagent, ≥99% •  • A4418 • for molecular biology, ≥99.0% (Sigma)

(Sigma) • • pricing • SDS • • S9378 • ≥99.5% (GC) (Sigma) • • pricing • SDS • • S0389 • for molecular biology, ≥99.5% (GC) (Sigma) • • pricing • SDS • • 84100 • for microbiology, ACS reagent, ≥99.0% (Sigma-Aldrich)		• • pricing • SDS • • RES1427A-A7 • PharmaGrade, NF, Manufactured under appropriate GMP controls for pharma or biopharmaceutical production. (Sigma) • • pricing • SDS • • A3920 • plant cell culture tested, ≥99.0% (Sigma) • • pricing • SDS • • 09978 • BioUltra, ≥99.0% (T) (Sigma) • • pricing • SDS • • A2939 • BioXtra, ≥99.0% (Sigma-Aldrich)

$CuSO_4 \cdot 5H_2O$ 209198 Copper(II) sulfate pentahydrate ACS reagent, ≥98.0% $CuSO_4$ PHR1477 Copper Sulfate pharmaceutical secondary standard $CuSO_4 \cdot 5H_2O$	 • Synonym: Acetic acid sodium salt • ○ Linear Formula: CH_3COONa ○ Molecular Weight: 82.03 ○ CAS Number: 127-09-3 • • S2889 • anhydrous, for molecular	30 Product Results • | Match Criteria: Product Name, Description  Ethyl acetate • Synonym: EtOAc • ○ Linear Formula: $CH_3COOC_2H_5$ ○ Molecular Weight: 88.11 ○ CAS Number: 141-78-6 • • 270989 • anhydrous, 99.8% (Sigma-Aldrich)

31293 Copper(II) sulfate pentahydrate puriss. p.a., ACS reagent, reag. ISO, reag. Ph. Eur., 99-102% $CuSO_4 \cdot 5H_2O$ C8027 Copper(II) sulfate pentahydrate BioReagent, suitable for cell culture, ≥98% $CuSO_4 \cdot 5H_2O$ C3036 Copper(II) sulfate pentahydrate plant cell culture tested, ≥98%	biology, ≥99% (Sigma-Aldrich) • • pricing • SDS • • 1.06264 • Sodium acetate anhydrous 99.99 Suprapur®. CAS No. 127-09-3, EC Number 204-823-8., anhydrous 99.99 Suprapur® (EMD Millipore)	• • pricing • SDS

alibaba.com purety material

FOB ***Reference*** *Price:Get Latest Price* *US $3.8-4.8 / Kilogram | 1 Kilogram/Kilograms (Min. Order)*  *Supply Ability:* *10000 Kilogram/Kilograms per Month* *Port:* *shang hai guangzhou or China main ports* Lactose monohydrate	 Peptone baceriological FOB **Reference** Price:Get Latest Price US $7.8-9.8 / Piece | 20 Piece/Pieces (Min. Order) Supply Ability: 10000 Kilogram/Kilograms per Month Port: Guangzhou,Shenzhen

peptone bacteriological

Shenzhen Taier Biotechnology Co., Ltd.

***US $11-20** / Kilogram*

***200 Kilograms** (Min. Order)*

Contact Supplier

Wuxi Accobio Biotech Inc.

US $1-15 / Kilogram

100 Kilograms (Min. Order)

Contact Supplier

Bacteriological peptone culture media

peptone powder

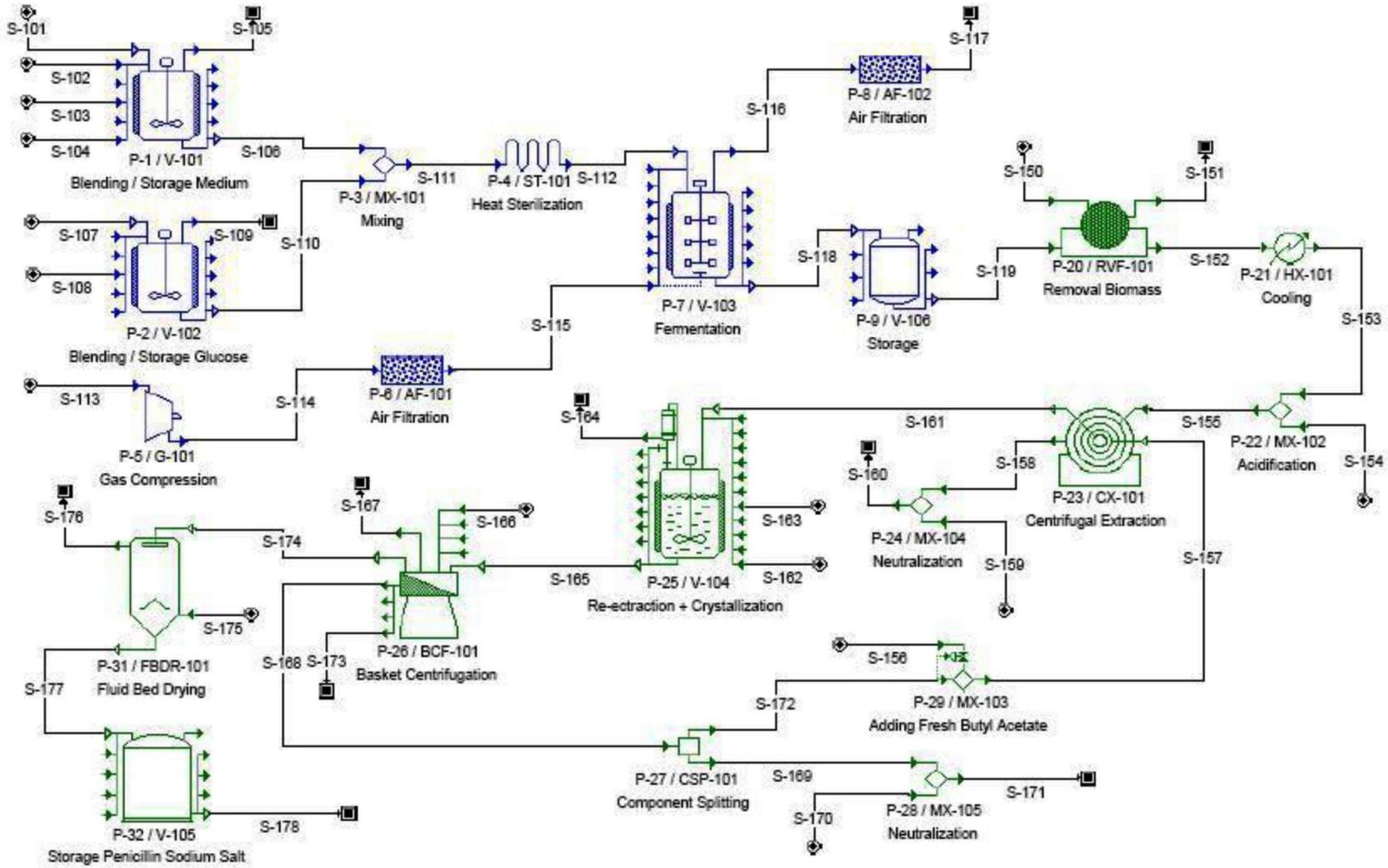
Guangdong Huankai Microbial Sci. & Tech. Co., Ltd.

US $9-12.5 / Piece

20 Pieces (Min. Order

2.4 Large Scale Penicillin Production

2.4.1 Process Flow Diagram



As in any bioprocess facility, there has to be an upstream and downstream process, the upstream processes in this case are refering to processes before input to the fermenter, while the downstream processes refers to the processes that are done to purify the output of the fermenter until it reaches to the desired product.

2.4.2 Medium for Penicillium

Medium preparation is necessary in bioprocesses which as it generally involve the use of microorganism to achieve their products. In the case of the Penicillium fungus, the medium usually contain its carbon source which is found in corn steep liquor and glucose. Medium also consist of salts such as Magnesium sulphate, Potassium phosphate and Sodium nitrates. They provide the essential ions required for the fungus metabolic activity.

Corn_steep_liquor.jpg

Corn steep syrup

2.4.3 Heat sterilisation

Medium is sterilse at high heat and high pressure usually through a holding tube or sterilse together with the fermenter. The pressurized steam is use usually and the medium is heated to 121°C at 30psi or twice of atmospheric pressure. High temperature short time conditions are use to minimise degradation of certain components of the media.

Sterilisation machine

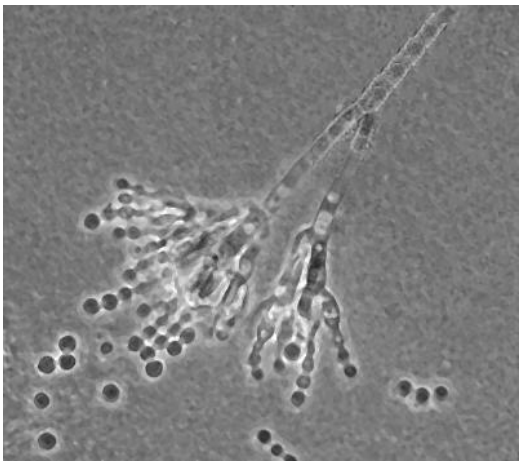
2.4.4 Fermentation

Fermentation for penicillin is usually done in the fed-batch mode as glucose must not be added in high amounts at the beginning of growth which will result in low yield of penicillin production as excessive glucose inhibit penicillin production. In addition to that, penicillin is a secondary metabolite of the fungus, therefore, the fed-batch mode is ideal for such products as it allows the high production of penicillin. The typical fermentation conditions for the *Penicllium* mold, usually requires temperatures at 20-24 °C while pH conditions are kept in between 6.0 to 6.5. The pressure in the bioreactor is usually much higher than the atmospheric pressure(1.02atm) this is to prevent contamination from occurring as it prevents external contaminants from entering. Sparging of air bubbles is necessary to provide sufficient oxygen the viability of the fungus. Depending on the volume of medium, for 2 cubic metres of culture, the sparging rate should be about 2.5 cubic metres per minute. The impeller is necessary to mix the culture evenly throughout the culture medium, fungal cells are much hardy and they are able to handle rotation speed of around 200rpm.

Fermenters.jpg

2.4.5 Seed culture

Like any other scale up process, usually the seed culture is developed first in the lab by the addition of *Penicillium* spores into a liquid medium. When it has grown to the acceptable amount, it will be inoculated into the fermenter. In some cases,the spores are directly inoculated into the fermenter.



The Penicillium fungus

2.4.6 Removal of biomass

Filtration is necessary at this point of the bioprocess flow, as bioseparation is required to remove the biomass from the culture such as the fungus and other impurities away from the medium which contains the penicillin product. There are many types of filtration methods available today, however, the Rotary vacuum filter is commonly employed as it able to run in continuous mode in any large scale operations. Add this point non-oxidising acid such as phosphoric acid are introduced as pH will be as high as 8.5. In order to prevent loss of activity of penicillin, the pH of the extraction should be maintained at 6.0-6.5.

Rotary_vacuum_filter.jpg

2.4.7 Adding of solvent

In order to dissolve the penicillin present in the filtrate, organic solvents such as amyl acetate or butyl acetate are use as they dissolve penicillin much better than water at physiological pH. At this point, penicillin is present in the solution and any other solids will be considered as waste.

solvent.jpg

Amyl Acetate as Solvent

2.4.8 Centrifugal extraction

Centrifugation is done to separate the solid waste from the liquid component which contains the penicillin. Usually a tubular bowl or chamber bowl centrifuge is use at this point.The supernatant will then be transferred further in the downstream process to continue with extraction.

disk_centrifuge.jpg

Disk centrifuge- One of the most common type of centrifuge for large scale production

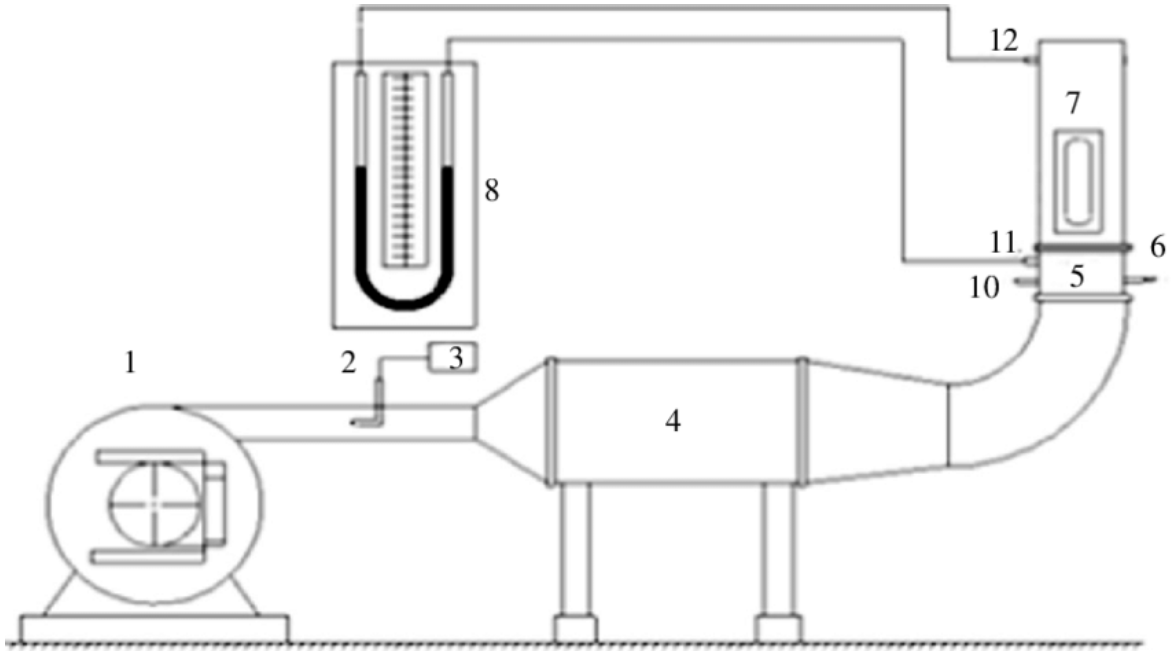
2.4.9 (Batch) Extraction

Penicillin dissolve in the solvent will now undergo a series of extraction process to obtain better purity of the penicillin product. The acetate solution is first mixed with a phosphate buffer, followed by a chloroform solution, and mixed again with a phosphate buffer and finally in an ether solution. Penicillin is present in high concentration in the ether solution and it will be mixed with a solution of sodium bicarbonate to obtain the penicillin-sodium salt, which allow penicillin to be stored in a stable powder form at room temperature. The penicillin-sodium salt is obtained from the liquid material by basket centrifugation, in which solids are easily removed.

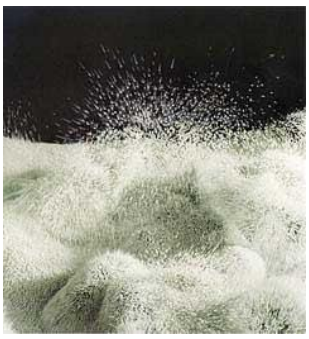
	
Batch extraction unit	Basket Centrifuge- Extremely using in the removal of solids in this case Penicillin salt

2.4.10 Fluid bed drying

Drying is necessary to remove any remaining moisture present in the powdered penicillin salt. In fluid bed drying, hot gas is pump in from the base of the chamber containing the powdered salt inside a vacuum chamber. Moisture is then remove in this manner and this result in a much drier form of penicillin.



Schematic diagram of the fluidized bed dryer: (1) blower, (2) pitot tube, (3) differential pressure transmitter, (4) electrical heater, (5) plenum chamber, (6) distributor, (7) drying chamber, (8) differential manometer, (9) humidity measurement sensor, (10) temperature control sensor, and (11, 12) pressure tap



spray_powder.jpg

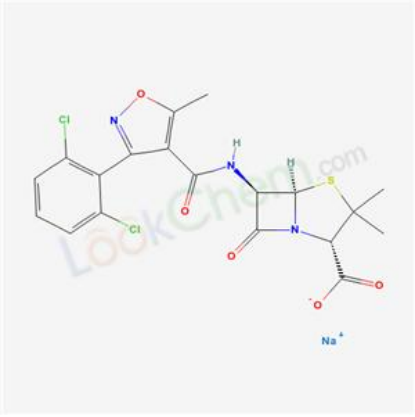
Powdered penicillin being blowned by hot air

2.4.11 Storage

Penicillin salt is stored in containers and kept in a dried environment. It will then be polished and package into various types of products such as liquid penicillin or penicillin in pills. Dosage of the particular penicillin is determined by clinical trials that are done on this drug.

Penicilin_sodium.jpg

The White Penicillin-Sodium salt



Chemical Structure of the Penicillin Sodium Salt

Chemical Structure of the Penicillin Sodium Salt

http://slideplayer.com/slide/10446753/"EXTRACTION & PURIFICATION of PENICILLIN

2.5 *العفن الذي أنقذ العالم و طريقة صنع البنسلين*

لأحد, 27 يناير 2013

- قطعة من الخبز أو قشر الحمضيات
- دورق مخروطي 750 مل
- وسيط (انظر للخطوة الرابعة)
- 1 لتر مخبار مدرج Éprouvette graduée
- عدد من زجاجات الحليب النظيفة ..

خطوات العمل :

1- لتحضير البنسلينيوم : تُعرّض قطعة من الخبز أو قشر الحمضيات لبيئة تكون درجة حرارتها 70 درجة فهرنهايت (25 درجة مئوية) و ينبغي أن يكون العفن أزرق أو أخضر .

2- لتعقيم الأدوات : ضع الدورق في فرن عند درجة حرارة 315 درجة فهرنهايت (157.2 درجة مئوية) على مدار الساعة ، أو تعقيم الأدوات في قدر الضغط لمدة لا تقل عن 15 دقيقة ، اغسل زجاجات الحليب جيداً .

3- ملء الدورق المخروطي : تقطع قطع الخبز أو قشر الحمضيات لقطع صغيرة و يملأ بها الدورق و نضعها بعد ذلك في الظلام عند درجة حرارة 70 درجة فهرنهايت (21.1 درجة مئوية) لمدة 5 أيام (فترة الاحتضان) ، بعد فترة الحضانة يمكن الإحتفاظ بالدورق في الثلاجة لمدة لا تزيد عن 10-14 يوم .

4- لتحضير الوسيط : أذب المكونات التالية حسب الترتيب المسرود في 500 مل من ماء الصنبور البارد

44,0 جرام لاكتوز أحادي الهيدرات , 25.0 جرام نشا الذره ,3,0 جرام نيتريت الصوديوم , 0.25 جرام كبريتات المغنيسيوم , 0.50 جرام فوسفات البوتاسيوم الأحادي , 2.75 جرام جلوكوز أحادي الهيدريد , 0.044 جرام كبريتات الزنك , 0.044 جرام كبريتات المنجنيز . ثم أضف أخيراً ماء الصنبور البارد لعمل لتر واحد . إستخدم حمض الهيدروكلوريك لضبط ال ph بين 5.0 و 5.5 .

5- ملء الزجاجات بمادة الوسيط : نملأ زجاجات الحليب بهذه الوسائط ، نستخدم عادةً كمية تكفي بحيث عندما نضع زجاجة بجانبها لا يصل هذا الوسيط إلى المكونات .

6- إضافة أبواغ البنسلين (العفن) : أولاً نقوم بتعقيم زجاجات الوسيط في قدر الضغط أو في الفرن كما فعلنا في الدورق المخروطي و عندما تبرد الزجاجات نضع بها ملعقة من أبواغ (عفن) الخبز أو قشر الحمضيات .

7- إحتضان الزجاجة : تترك الزجاجات للراحة بدون عائق في الجانبين عند درجة حرارة 70 درجة فهرنهايت (21.1 درجة مئوية) لمدة 7 أيام ، إذا تكوّن البنسلين سيكون الجزء السائل في الوسيط بعد هذه الفترة (الحضانة) ، و أخيراً تصفية الوسيط و تبريده على الفور ، إذا كان يجب استخدامه يستخدم في أقرب وقت ممكن و إن كان ينبغي تجنب ذلك .

لا ينبغي للبنسلين المتكون من هذه التجربة أن يستخدم إلا إذا كان لغرض البقاء أو استخدامات أخرى
فمن الممكن للعفن السام أن ينمو جنباً إلى جنب مع البنسلين ، حتى لو كنت تعرف مالذي تقوم به فمن الممكن لمتبطات نمو العفن وقف نمو أبواغ البنسلين .

2 http://chemi101.blogspot.com/2013/01/blog-post.html

2.6 Sabouraud Agar

Agar Sabouraud agar in a Petri dish with a colony of Trichophyton rubrum var. rodhaini.

Sabouraud's agar (which is named after Raymond Sabouraud) is an isolation medium for Fungi (molds and yeasts).

It was created by, and is named after, Raymond Sabouraud in 1892. Later adjusted by Chester W. Emmons when the pH was brought closer to the neutral range and the dextrose concentration lowered to support the growth of other fungi. The pH of 5.6 of the traditional sabouraud agar inhibits bacterial growth.

(Dermatophyte_test_medium&action)

2.7 Uses of Ethyl acetate

Ethyl acetate is used in the following areas:

• solvent to remove nail polish (called solvent);

• solvent for dangerous glues to "sniff" because it causes a feeling of intoxication that can damage the brain;

• solvent for nitrocellulose;

• produce to decaffeinate coffee beans and tea leaves;

• solvent for chromatography mixed with a non-polar solvent such as hexane;

• solvent for extractions (antibiotics);

2.7.1 Synthesis of Ethylacetate

Ethyl acetate is synthesized by the Fischer esterification process, resulting from a reaction between acetic acid and ethanol. An acid, such as sulfuric acid, catalyzes the reaction. CH3CH2OH + CH3COOH → CH3COOCH2CH3 + H2O.

2.8 Revelation of efficacite to penicillin

2.8.1 LBmedium

The aim of the culture to tested the penicillin soluble

Preparation of medium

they are called the two main bacteria of yogurt *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.

Pour les articles homonymes, voir LB.

LB culture medium in a bottle and in a culture dish. The LB culture medium (For lysogeny broth or incorrectly Luria-Bertani medium) is a nutrient culture medium, initially used for bacterial culture1. It was first developed by Bertani, who named it lysogeny broth (lysogenic broth) in its first publication2. LB media have become an industry standard for culturing

Escherichia coli since the 1950s. They have been used extensively in molecular microbiology for the preparation of DNA plasmids and recombinant proteins. It remains to this day, one of the most used environments for the maintenance and culture of recombinant lines of Escherichia coli. There are various compositions of LB. Although they are different, they usually share some of the common components they have to support the growth of species in culture. • Peptides and peptones of casein• Vitamins (Vitamin B included) • trace elements (eg nitrogen, sulfur, magnesium) • Minerals

2.9 Bactéris of yaourt

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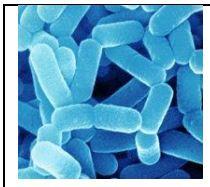
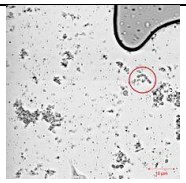
<tr>
<td>  </td>
<td>  </td>
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<td>Lactobacillus delbrueckii subsp. Bulgaricus

is a microorganism of the genus Lactobacillus. It is a gram positive bacillus. His discovery was due to the Bulgarian student of medicine Stamen Grigoroff (in) in 19051, and named in 1919, Thermobacterium bulgaricum, by the Danish Orla Sigurd Jensen (da) (1870-1949). From 1971 to 1983, its name was Lactobacillus bulgaricus, renamed by Morrison Rogosa and Danish Poul Arne Hansen (1902-1972).

Caractéristiques:
- gram +
- anaérobie
- catalase –
- oxydase –</td>
<td>The thermophilic streptococcus(or Streptococcus thermophilus1,2)

is a thermophilic food bacterium (growth optimum at 43 ° C), present only in the fermentation of milk, where it is used in particular in association with the bacterium Lactobacillus delbrueckii subsp. bulgaricus for making yoghurt.
• as a cocci (rounded shell), 0.7-1 µm, forming strings or pairs
• with positive Gram stain
• its optimum growth temperature is between 37 ° C and 60 ° C, depending on the strain. Does not grow at 15 ° C but all strains grow at 45 ° C and most at 50 ° C
• strict homofermentative bacterium (producing L-lactate), microaerophilic
• non-pathogenic

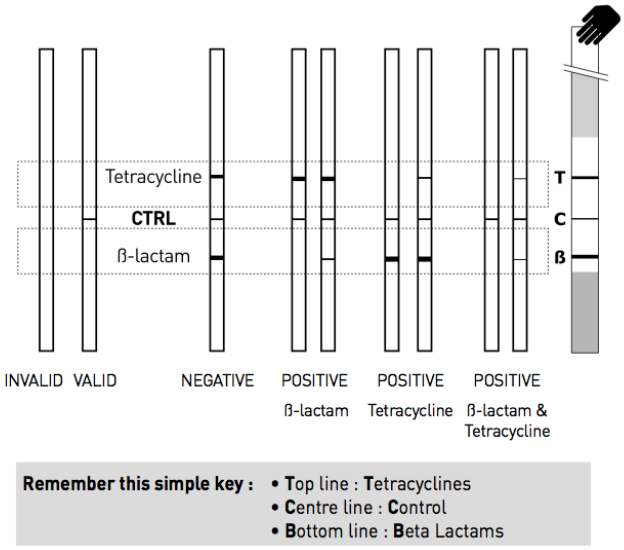
its cultivation requires B vitamins and some amino acids.</td>
</tr>
<tr>
<td colspan="2">In Gram-positive bacteria, the different β-lactams reach the transpeptidases through the already formed or in-process peptidoglycan wall. In contrast, in Gram-negative bacteria, they only reach these enzymes after penetration through the pores of the outer membrane</td>
</tr>
</table>

2.9.1 Twin sensor

The test requires the use of two components. The first component is a microwell containing predetermined amounts of receptors and antibodies bound to gold particles. The second is a gauge composed of a set of membranes with specific capture lines.

For a valid test, the red control line should be visible after the second incubation. Both ether are the specific test lines placed on both sides of the control line. The line of β-

lactam antibiotics [penicillins and cephalosporins] is located under the "control" whereas the tetracycline-related line is located above. When the reagent from the microwell is resuspended with a milk sample, the two receptors will bind the corresponding analytes if they are present during the first 3 minutes of incubation at 40 ° C. Then, when the dipstick is immersed in the milk, the liquid begins to run vertically on the gauge and passes through the catchment areas.

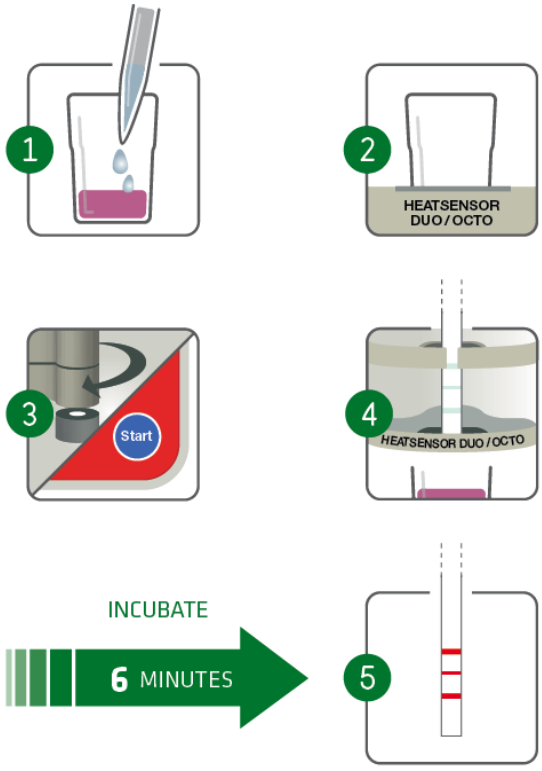


When the sample is free of antibiotics, color development occurs at the specific capture lines, indicating the absence of targeted analytes in the milk sample. On the contrary, the presence of antibiotics in the sample will not cause the appearance of the colored signal at the specific capture lines.

Beta-lactams and tetracycline antibiotics are the antibiotics most commonly used in the treatment of bacterial infections in dairy cattle. A specific indication for administering both types of antibiotics is infectious mastitis. These drugs are also administered to animals in foods for the promotion of growth and for collective prophylaxis.

The monitoring of beta-lactams and tetracyclines in milk is important because of the hypersensitivity of certain individuals to these antibiotics and the emergence of bacterial strains resistant to antibiotics. In addition, the overall residual level of antibiotics could alter the efficiency of industrial processing from raw milk to the preparation of cheese or other fermented dairy products.

Maximum Residue Limits (MRLs) have been specified for food products and milk to control the levels of these antibiotics reaching the consumer. The kit is available in a version specific to the European Union Maximum Residue Limits (KIT020).



5 http://www.intermed.be/fr/produits-professionels/laboratoire-diagnostiques/produits-laitiers/twinsensor.html

2.9.2 Analysis of Penicillin purity: ELISA Kit

www.abnova.com:

Catalog Number KA3305
96 assays
Version: 03
Intended for research use only
KA3305 3 / 9

During routine testing of milk samples for antibiotics, in more than 90% of the positive cases, betalactam preparations or penicillins are detected. The method of choice for the determination of penicillin contamination in food has always been a microbiological assay. These procedures allow however no quantitative determination and no identification of the antibiotic drug, which is achieved by a sensitive ELISA test kit or immunoaffinity columns together with HPLC. Principle of the Assay

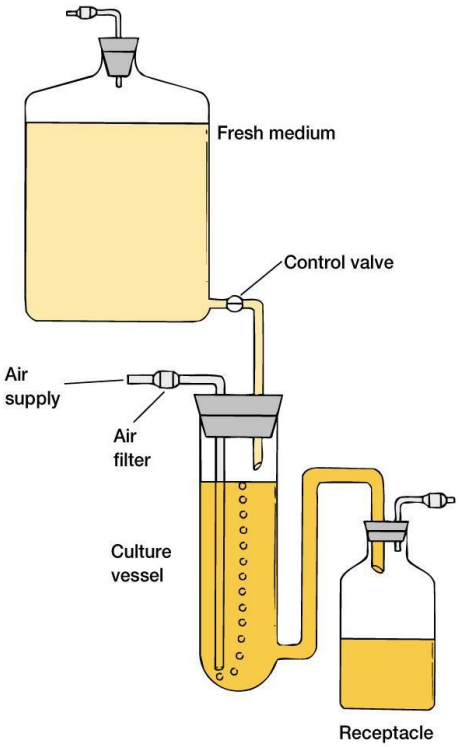
The Penicillin ELISA Kit is based on the principle of the enzyme linked immunosorbent assay.

A penicillin conjugate is bound on the surface of a microtiter plate. A penicillin conjugate is bound on the surface of a microtiter plate. Penicillin containing samples or standards and an antibody directed against penicillin are given into the wells of the microtiter plate. Immobilized and free penicillin compete for the antibody binding sites. After one hour incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A peroxidase conjugate directed against the penicillin antibody is given into the wells and after another hour incubation, the plate is washed again. Then a substrate solution is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured photometrically at 450 nm. The concentration of penicillin is indirectly proportional to the color intensity of the test sample.

2.10 Le chémostat

- Rythme d'introduction du milieu stérile = rythme d'élimination du milieu.

- Un élément nutritif essentiel est fournit en quantités limitées (i.e. un acide aminé



Mold culture Penicillium chrysogenum in liquid Sabouraud medium, with gentle agitation.

It is noted that, unlike bacteria that develop in a liquid medium without forming colonies mildew by clouding the medium, molds form spherical structures (due to their centrifugal growth from a spore) and that the medium remains perfectly limpid (a disorder of the environment thus translating a microbial contamination)

3_image: http://droguet-sebastien.e-monsite.com/medias/images/penicillium-sabouraud-liquide-1-.jpg?fx=r_1200_800

Reference: http://droguet-sebastien.e-monsite.com/pages/activites-technologiques-terminale-2014-2015/at03-etude-des-mycetes.html

2.11 Principles of pO2 Measurement with the Clark Electrode

The Clark Oxygen Electrode

The principles of amperometric oxygen measurement are discussed at some length in the chapter on the platinum oxygen cathode.

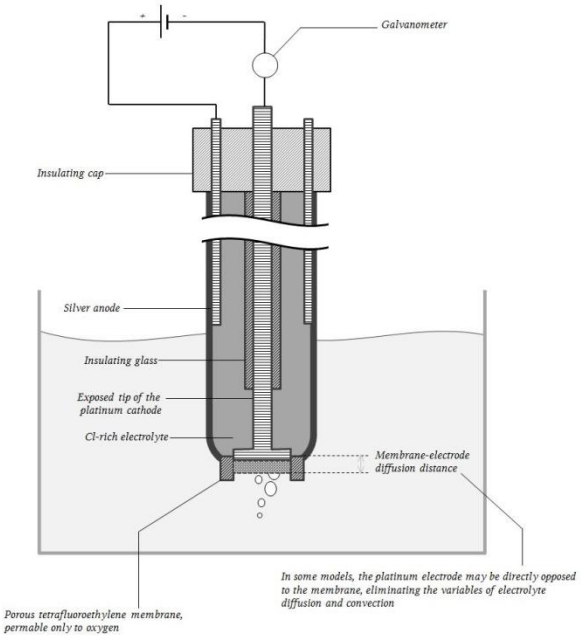
In brief:

- A silver anode and platinum cathode are suspended in an electrolyte.
- Oxygen is dissolved in the electrolyte.
- A voltage of known magnitude (about 700 mV) is applied to the electrodes.
- Oxygen is reduced at the cathode and silver is oxidised at the anode.

- The resulting current increases as the voltage increases.
- The current reaches a plateau when the rate of reaction is determined by the diffusion of oxygen rather than the voltage.
- This plateau correlates to the oxygen tension in the electrolyte.

The major difference between this electrode and the earlier oxygen cathode is the addition of an oxygen-permeable membrane. Something resembling the original patent application diagram can be found here.

Its butchered representation can be found below.



Reference

derangedphysiology.com/main/core-topics-intensive-care/arterial-blood-gas-interpretation/Chapter 2.0.5/principles-po2-measurement-clark-electrode

2.12 Uses of Ethyl acetate

Ethyl acetate is used in the following areas:

• solvent to remove nail polish (called solvent);

• solvent for dangerous glues to "sniff" because it causes a feeling of intoxication that can damage the brain;

• solvent for nitrocellulose;

• produce to decaffeinate coffee beans and tea leaves;

• solvent for chromatography mixed with a non-polar solvent such as hexane;

• solvent for extractions (antibiotics);

Summary

Ethyl acetate is synthesized by the Fischer esterification process, resulting from a reaction between acetic acid and ethanol. An acid, such as sulfuric acid, catalyzes the reaction. CH3CH2OH + CH3COOH → CH3COOCH2CH3 + H2O.

Since this reaction is reversible and produces a chemical equilibrium, the yield is low unless the water is removed. In the laboratory, ethyl acetate can be separated from water using the Dean-Stark process.

2.13 Synthesis of ethyl acetate

synthesis of ethyl acetate.html https://www.youtube.com/watch?v=cFxZ0NircIk

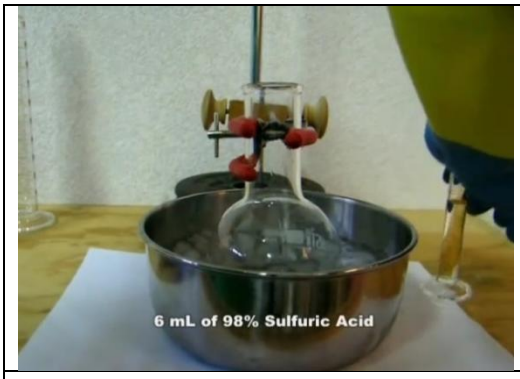
30ml acetic acid

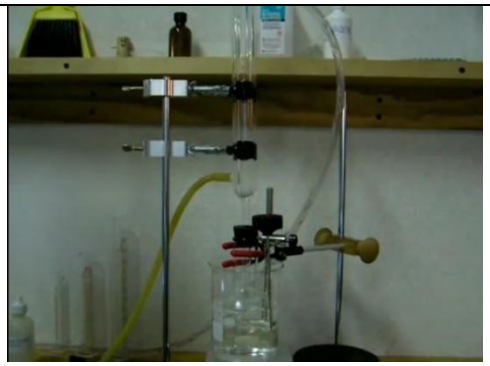
30ml ethanol

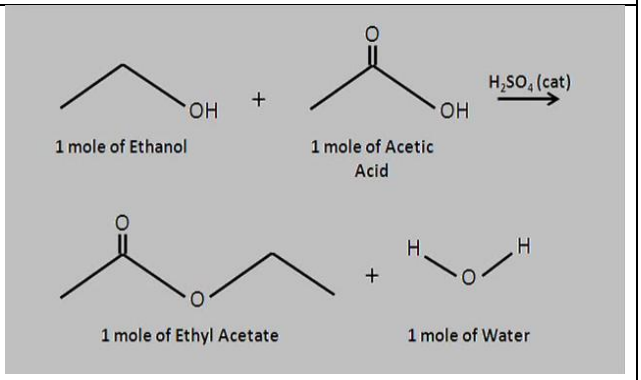
6ml sulfuric acid

3g Na2co3+15ml H2O

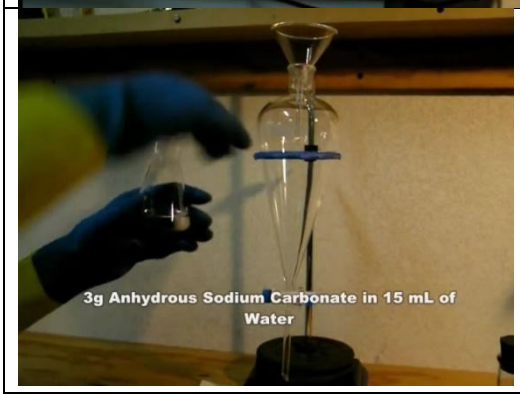
3g CaCL2+15ml H2O

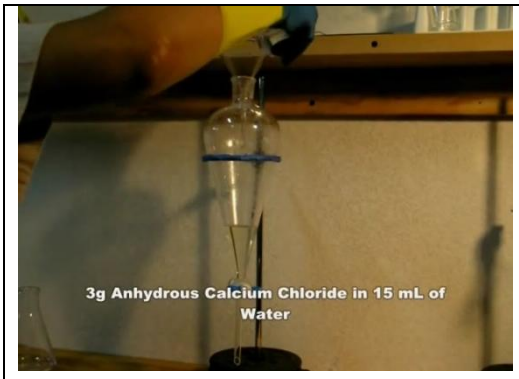

6 mL of 98% Sulfuric Acid




OH
+
OH
H_2SO_4 (cat)
1 mole of Ethanol
1 mole of Acetic Acid
O
O
+
H
O
H
1 mole of Ethyl Acetate
1 mole of Water


تم النسخ إلى الحافظة


3g Anhydrous Sodium Carbonate in 15 mL of Water







2.14 Dean Stark's device

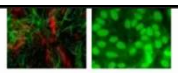
The device is generally used for azeotropic distillation. For example, to remove water produced by a reaction involving toluene. A heteroazeotropic mixture of toluene and water evaporates from the flask, but only toluene returns (being of lower density) since it floats above the water which accumulates in the "burette".

For example in the case of the esterification of butanol with acetic acid catalyzed by sulfuric acid. The vapors contain 63% ester, 24% water and 8% alcohol; after condensation, the organic phase which returns to the medium contains 86% of ester, 11% of alcohol and 2% of water while the aqueous phase consists of 97% pure water)

2.15العمل مع المجهر (working with microscope)

OBJECTIVE POWER	4X, 10X, 40X, 100X
OBJECTIVE SIZE	N/A
EYEPIECE POWER	10X, 25X
EYEPIECE SIZE	N/A
EYEPIECE FOCUSABILITY	N/A
CAMERA	N/A
CAMERA PIXEL	N/A
CAMERA SENSOR	N/A
REDUCTION LENS	N/A
STAGE	N/A
MICROSCOPE STAND	No
LIGHTING CONFIGURATION	Transmitted

PRODUCT CODE	5555268
MICROSCOPE TYPE	Compound
SPECIALIZED	EPI-Fluorescenc
APPLICATION	Clinic, Veterinary, Laboratory
MAGNIFYING TYPE	Multi-Power
MAGNIFICATION POWER	40X to 2500X
OPTICS	Achromatic
FIELD VIEW	N/A
HEAD TYPE	Trinocular
OBJECTIVE POWER	4X, 10X, 40X, 100X



$1,619.99

IN STOCK

- Epi-fluorescence illumination with blue and green exciting light filters
- Transmitted brightfield illumination
- Trinocular compensation free viewing head, easy to mount digital camera to catch ordinary and high contrast fluorescent images
- 4 high quality fluorescence objectives: FLUOR 4x, 10x, 40x(S), 100x(S,Oil)
- Large stain-resistant double layer stage can hold two slides in parallel

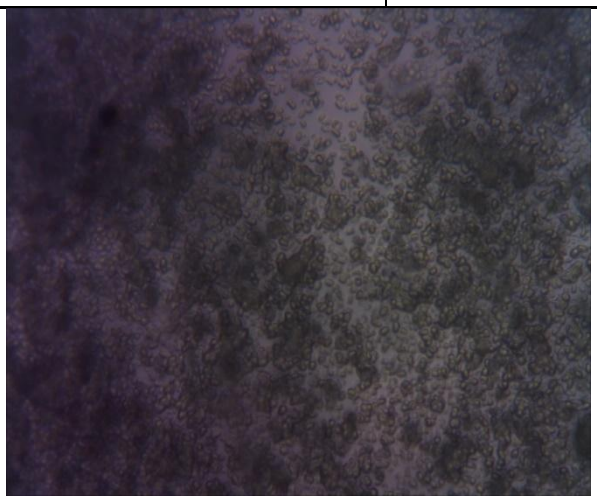
Qty: 1 ADD TO CART

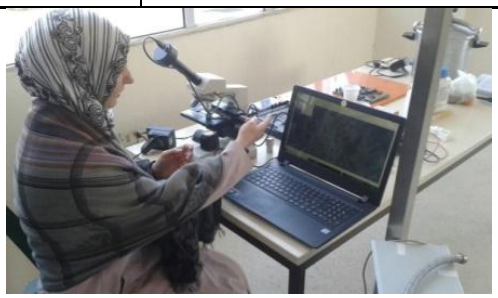
Add to Wishlist | Add to Compare

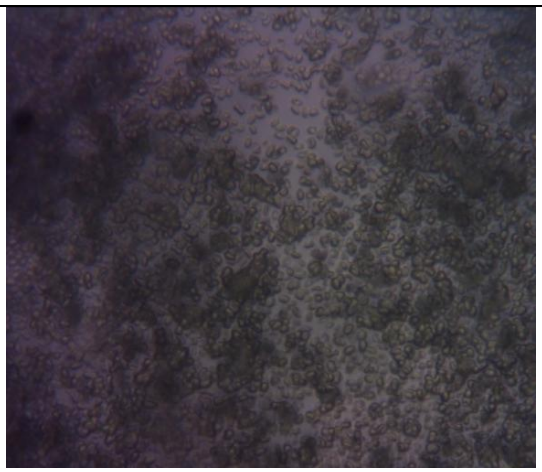
	N / A
نوع المكثف	N / A
بصريات مكثفة	N / A
بارلو لينس	N / A
قزحية	N / A
مزود الطاقة	N / A
التعليم	N / A
اسم العلامة التجارية	OMAX
اللون	N / A

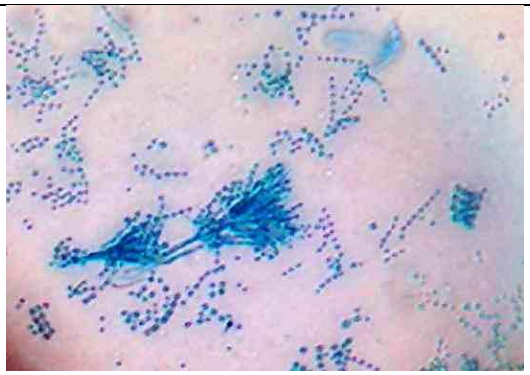
نوع خفيف	الهالوجين ، بخار الزئبق
قوة الضوء	N / A
شكل خفيف	N / A
عدد من المصابيح الكهربائية	2
المرحلة DIV	N / A
مكثف	N / A
نوع المكثف	N / A
بصريات مكثفة	N / A
بارلو لينس	N / A
قزحية	N / A
مزود الطاقة	N / A
التعليم	N / A

Your search - **microscope fluorescent (YJ-1006285)** - did not match any





Techniques	Aspect microscopique (G×40)	Caractères microscopiques
Adhesive tape A small piece of tape is applied by the sticky face on the colony then deposited on a slide. Then observation under immersion microscope: the goal (× 40) then to (× 100) (Joffin, 2013)		Spores

Lactophenol blue cotton A fragment of the colony is removed with the help of a platinum loop and deposited on aslide in a dye drop afterwards cover with a coverslip-object that makes the preparation crushed (Chabasse et al., 2002		-Conidiophores isolés -Pénicilles constitués de phialides branchés directement à l'extrémité du conidiophore

Since this reaction is reversible and produces a chemical equilibrium, the yield is low unless the water is removed. In the laboratory, ethyl acetate can be separated from water using the Dean-Stark process.

2.16 Preparation of medium saboureu

2.16.1 Experimental protocol

	g		ml		g	ml	
	15		1000				250
	20		1000				
	10		1000				
glucose	2		100	eau	0.5	25	5
peptone	1		100		0.25	25	2.5
agar	1.5		100		0.375	25	

Materials:

Becher 100ml, Stirrer
Erlenmeyer, Petri dish, Libra
Glucose, Microbiological medium agar, Tryptone yeast extract, KH_2PO_4, $MgCl_2$, $CaCl_2$, distilled water, autoclave, charcoel treatment, acetic acid, ethanol, amino acid (instead of peptone)

Microwave or Bunsen burner and penicillium orange (green spot)
Incubator

Procedure:

The glassworks are washed with tap water and then with distilled water and sterilized the glassworks by the autoclave
0.5 g of glucose, 0.25 g of tryptone, 0.4 g of agar and 0.25 g of KH 2 PO 4, 0.25 g of MgCl 2 are weighed into the Erlenmeyer flask using a pipette and 0.25 ml of CaCl 2 are measured.

The test solution is filled with 25 ml of water and poured into the Erlenmeyer flask.
We put the Erlenmeyer on the magnetic stirrer at 100 °C until two minutes left to cool a little
Pour the mixture into the semi-covered dough box until the solidified solid (gel)
We put some spore of the green spot on the gel obtained we semi cover and put it in the incubator 48 h, we read
After 48h and reading the box the penicillium and ready to grow in a liquid medium

2.17 Preparation of ethyl acetate

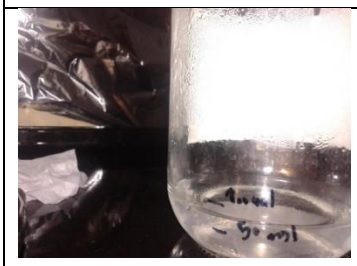
Synthesis:

Ethyl acetate is synthesized by the Fischer esterification process, resulting from a reaction between acetic acid and ethanol. An acid, such as sulfuric acid, catalyzes the reaction. $CH_3CH_2OH + CH_3COOH \rightarrow CH_3COOCH_2CH_3 + H_2O$.

Since this reaction is reversible and produces a chemical equilibrium, the yield is low unless the water is removed. In the laboratory, ethyl acetate can be separated from water using the Dean-Stark process 200 ml vinegar is placed in an Erlenmeyer flask and heated to boiling to evaporate the water after cooling. 100 ml of ethanol are placed in an Erlenmeyer flask and the slowly cooled reaction is added.

To increase the yield, the technique of

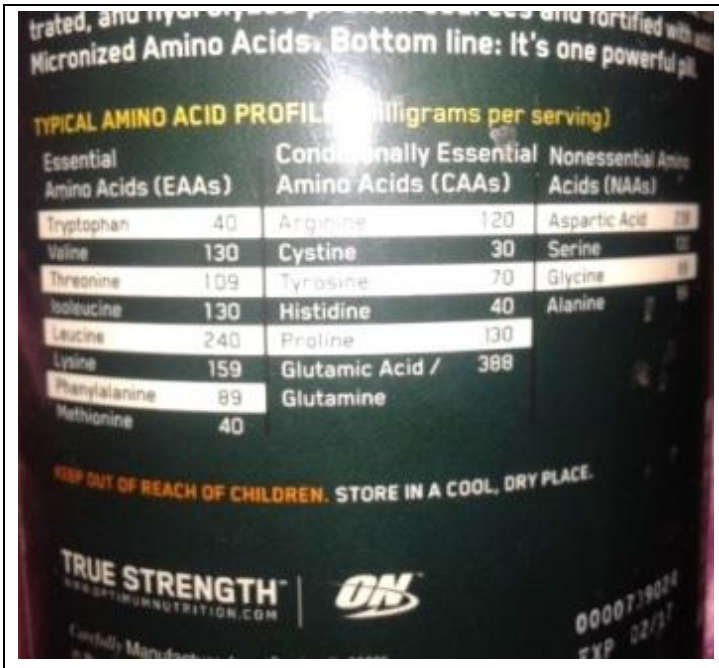
Warming of vinegar

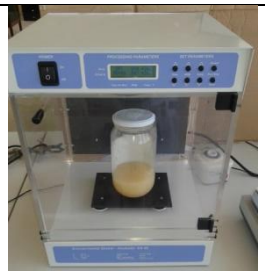


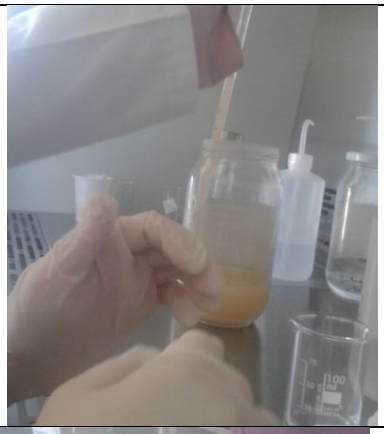
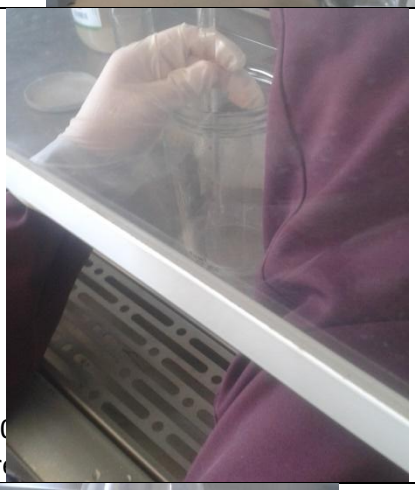
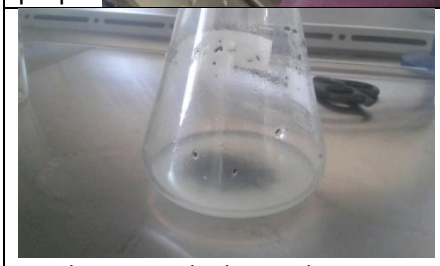
Ethyl acetate

3 Experimental Laboratory scale production of penicillin

3.1 Experiment 1: Synthese of penicillin by Amino acids

<table>
<tr>
<td> 

Material:
amino+ Table sugar, milk poudre (lactose), eau</td>
<td> 
Incubation of liquid medium in the incubator at 26 0c + chaker</td>
</tr>
<tr>
<td> 
</td>
<td>After fermentation of content
Amino sugar and table salt, lactose (lacto milk) water and qlq penicillium spore</td>
</tr>
</table>

take 10ml 	It is also pu 
Take 1(prepar 	It is also 
 incubation with charcoal treatment (0.43g)	Then we filter we put the contents in a tube (10ml) we incubate it is desired that the contents contain soluble penicillin
	Tube content of liquid it is desired that the contents contain soluble penicilli

3.1.1 Culture of bacteria of yogurt bacteria+ penicillin

The aim of the culture to tested the penicillin soluble

Preparation of medium

they are called the two main bacteria of yogurt *Lactobacillus bulgaricus* and *Streptococcus thermophilus.*

How long does it take for them?

About 20 minutes. Do you imagine how many twins this will give

it if we all divide 3x per hour!

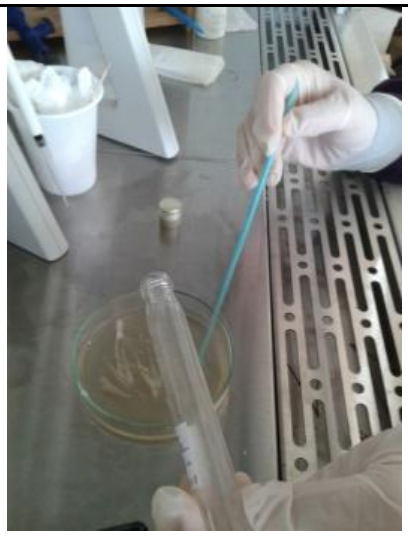
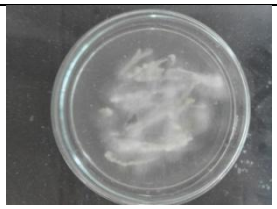
Materials

petri dish, yoghurt, Nacl, tripton yeast extract agar, distilled water, magnetic stirrer, Bunsen burner, wooden cord, handle, flame.

Protocol : work under high The glassworks are washed with tap water and then with distilled water and sterilized the glassworks by the autoclave
Water in a 250 ml beaker and put the tube of tryptone to melt the contents
After adding 10 ml of water in the tube after homogenization is poured into the Erlenmeyer flask.
We put the Erlenmeyer on the magnetic stirrer at 100 ○ C until two minutes left to cool a little
Pour the mixture into the semi-covered dough box until the solidified solid (gel)
We put yogurt on the gel obtained and put it in the incubator for 48 hours, we read.

1 tube de tryptone		
0.5g Nacl		
10 ml eau distillee		

0.5gNaCl water10ml Becher containing water to warm the tube of tryptone

<table>
<tr><td>  </td><td>  </td></tr>
<tr><td> 
spread of yoghurt</td><td> 
spreading penicillin</td></tr>
<tr><td>  </td><td>After incubation from 26.4.2018 until 28.4.2018</td></tr>
</table>

3.2 Experiment 2: Preparation of penicillium colony

<table>
<tr><td>  </td><td>  </td></tr>
</table>

weigh glucose

sterilization of glassware

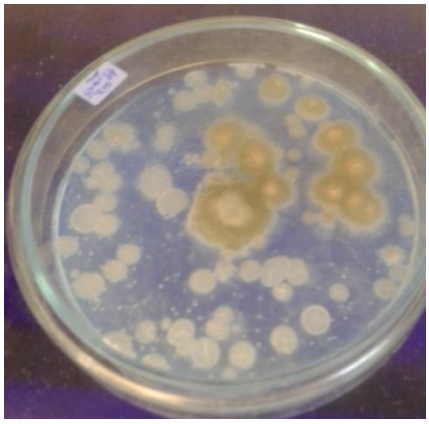
heat the tube tryptone agar yeast extract

prepare the petri dish 19-04-2018

25-4-2018

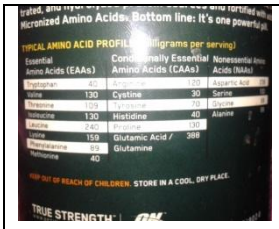
3.3 Experiment 3: Preparation of penicillin cristal by amino acids

Uv machine

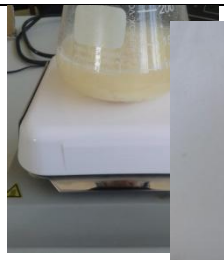


UNDER UV observation blue toxin secreted by penicillium

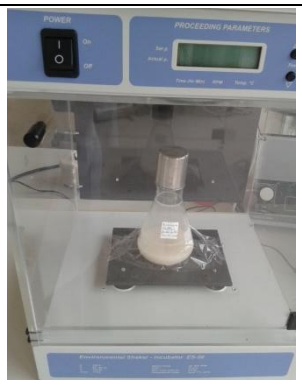
Materials used for the manufacture of liquid medium:
2g glucose + 2g lactose (milk) + 1g Amino
0.1g MgCl2 + 0.1g kcl + 0.5g KH2PO4 +
100ml distilled water



Amino acids



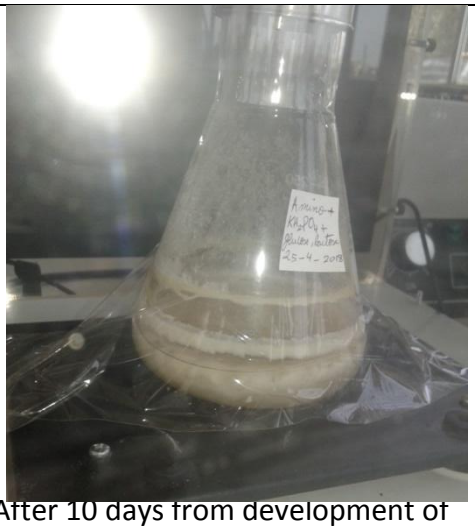
25-04-2018



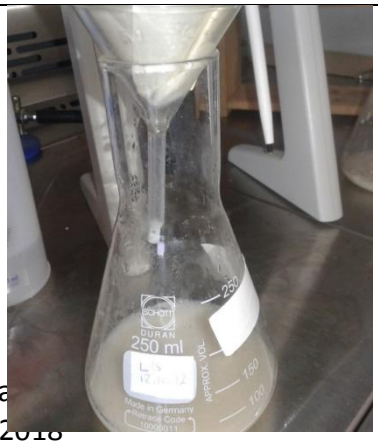
incubation

0.43 g charcoal treatment+ 0.5g KH2PO4

acid such as phosphoric acid are introduced as pH will be as high as 8.5. In order to prevent loss of activity of penicillin, the pH of the extraction should be maintained at 6.0-6.5.

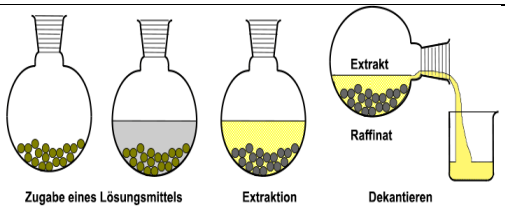


After 10 days from development of *penicillium*



Filtra

4-5-2018



Charcoal treatment incubation
+KH2PO4 to regulate the pH

Inc acétate (vinaigre +éthanol not pure) incubation in refrigerator

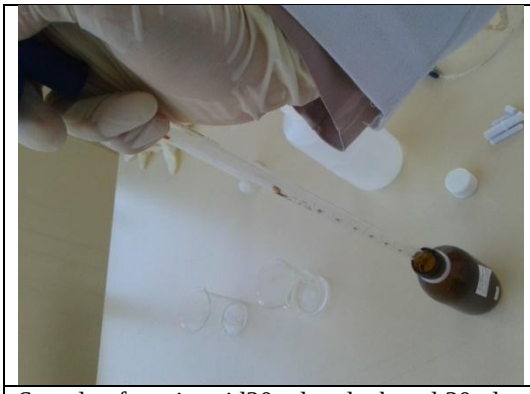
26-5-2018
Following the protocol we put 5g of sodium bicarbonate we note an effervescence
So the ethyl acetate that we prepare contains More vinegar which allows this result

Saturday, June 30, 2018 1:28 PM

long time incubation in the refrigerant after filtration obtaining penicillin crystals

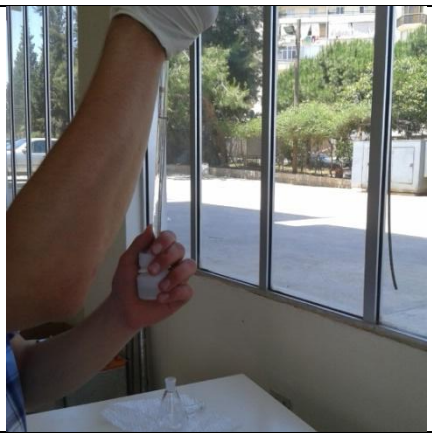
3.4 Experiment 4: Preparation of ethyl acetate

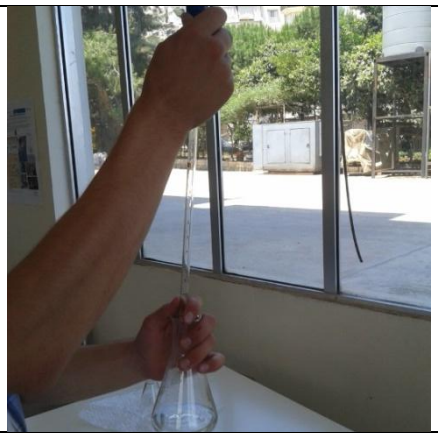
2-6-2018



Sample of acetic acid30 ml and ethanol 30ml

acetic acid prepared by heating the vinegar from 250ml to 15ml





Sample of sulfuric acid (37% acid +water) 17 ml

for the reaction to take place, the contents are heated with condensation

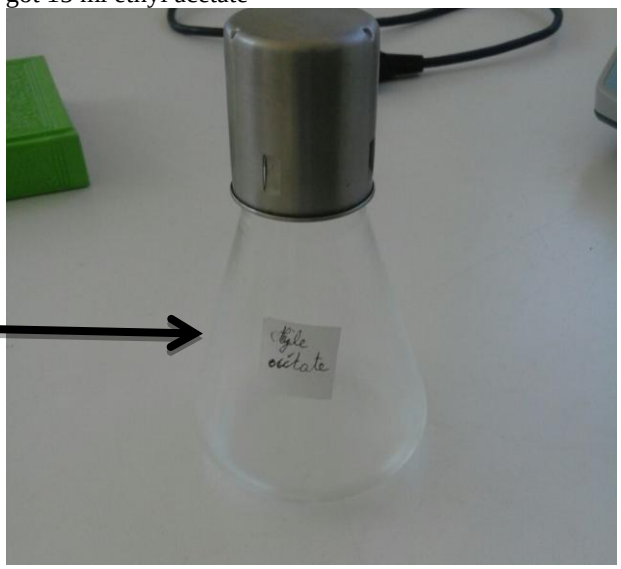
moun

Heated to 88 °C

the change of smell shows that ethyl acetate is formed

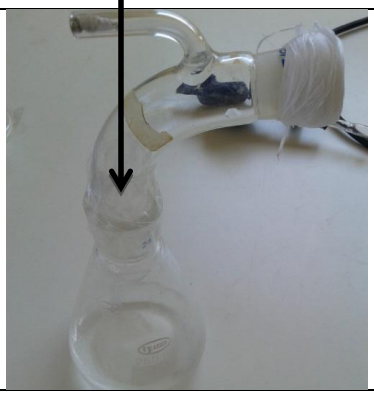
9-6-2018

Of 30ml acetic acid +30ml ethanol+17ml sulfuric acid we got 15 ml ethyl acetate



condensation of ethyl acetate 88° C

We tested that by sodium carbonate



	effervescence[2] This means that we have acetic acid (Because there was a reaction with pure sodium bicarbonate)	no effervescence This means that we **do not have** an acetic acid. There is pure ethyl acetate. (Because there was no reaction with pure sodium bicarbonate)

3.5 Experiment 5:Preparation of ethyl acetate with the spirit of vinegar

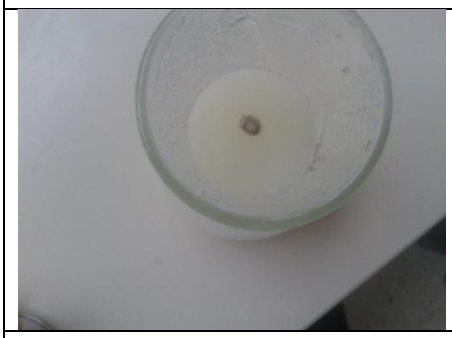
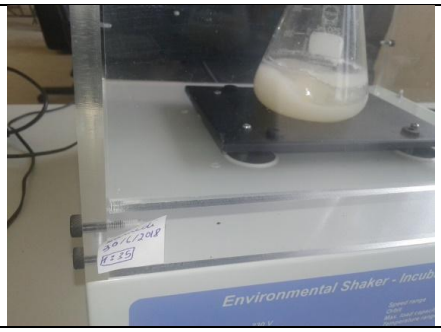
100ml sprint vinegar,100ml ethanol

higher yield of ethyl acetate

3.6 Experiment 6: Preparation of liquid medium with peptone

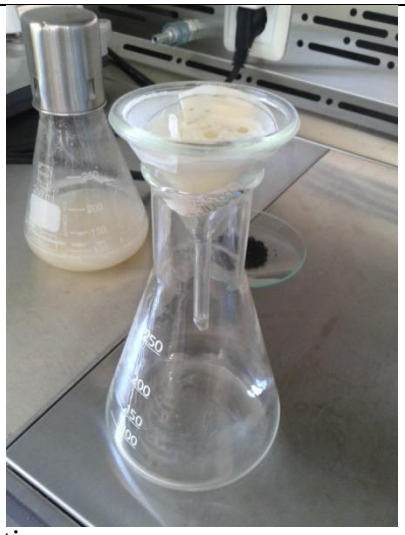
Le 30-6-2018	

[2] effervescence: escape of gas from an aqueous solution

 1g peptone,2g glucose ,2g lactose 0.1gKCl.,0.1gMgCl2,0.5g KH2PO4 ,100ml distilled water	
 7 days	 incubation

After 7 days Purification of penicillin

	 0.5 g



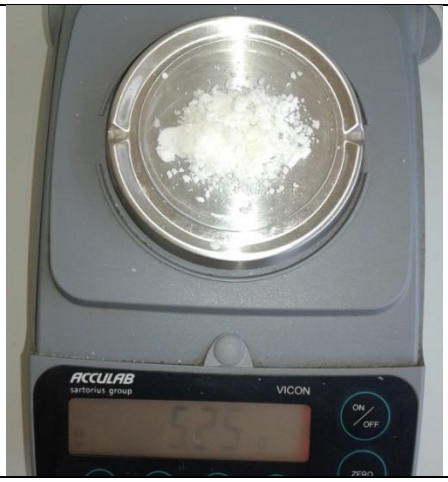
filtration

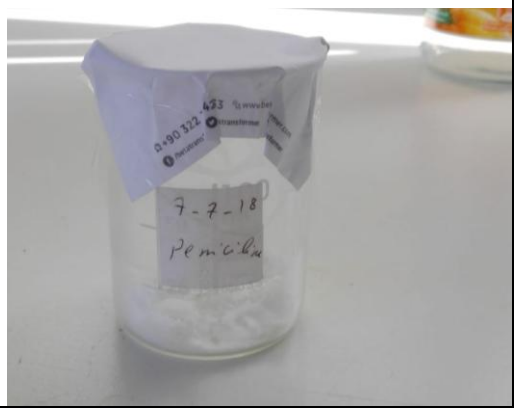
addition of pure ethyl acetat

remove the content from the tubes

centrefigation

From 100 ml of medium we obtained 5.25 g of penicillin





3.7 Some Experiments done once again[3]

3.7.1 Preparation of Agarose Gel

Materials:

-Tube of tryptone

-Beaker

-Erlenmeyer

-Distilled water

-Glucose

-Ethanol

[3] Samar Youssef, Report 10.12.2019

-Petri dish

-Gloves

-Spatula

-Lighter

-Heater

-Digital balance

-Graduated cylinder

Procedure:

- First step: we put an orange in a fermentation conditions until we become able to see a fermented region.

- Step 2: preparation of agarose gel:

1-we put the tryptone tube into a 250 ml beaker full of water

2- we heat the beaker using a lab heater until the gel melt

3-we measure 10 ml of water using a graduated cylinder

4- we weight 0.5 g of glucose powder using a digital balance.

5-we mix the Tryptone Gel, the Glucose and the Water in the Erlenmeyer.

We keep heating until we get a homogeneous mixture.

Then we fill the mixture in the petri dish.

And we wait around 30 mins until the gel become totally solidified.

Remark:

A plate which has been streaked showing the colonies thinning as the streaking moves clockwise.

	In microbiology, streaking is a technique used to isolate a pure strain from a single species of microorganism, often bacteria. Samples can then be taken from the resulting colonies and a microbiological culture can be grown on a new plate so that the organism can be identified, studied, or tested.

3.7.2 Preparation of liquid medium

Materials:

-Beaker

-Spatula

-Glucose

-Lactose

-Peptone

-MgCl2

-KCl

-KH2PO4

-Distilled water

-Erlenmeyer

-Metallic paper

-Ethanol or Ethyl alcohol

-Graduated cylinder

-Digital balance

-Magnetic hot plate stirrers

-Shaker.

Procedure:

-First step: sterilization.

We put 2 ml of distilled water in the Erlenmeyer we close it with metallic paper then we heat until the solution start boiling (so now T~100˚C).

-Step 2: preparation of liquid medium.

we weight: -2 g of Glucose powder.

-2 g of Lactose

-1 g of Peptone

-0.1 g of $MgCl_2$

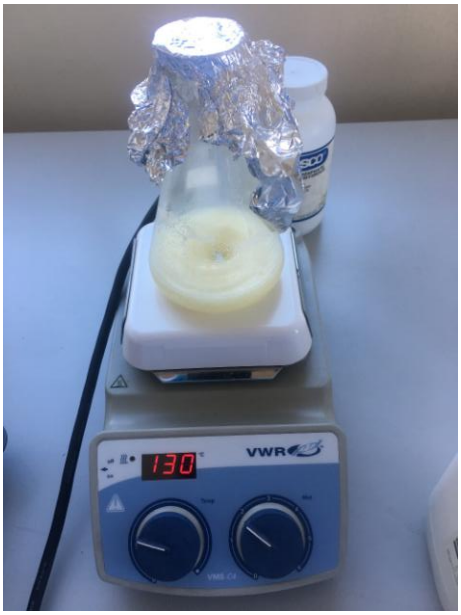
-0.1 g of KCl

-0.5 g of KH_2PO_4

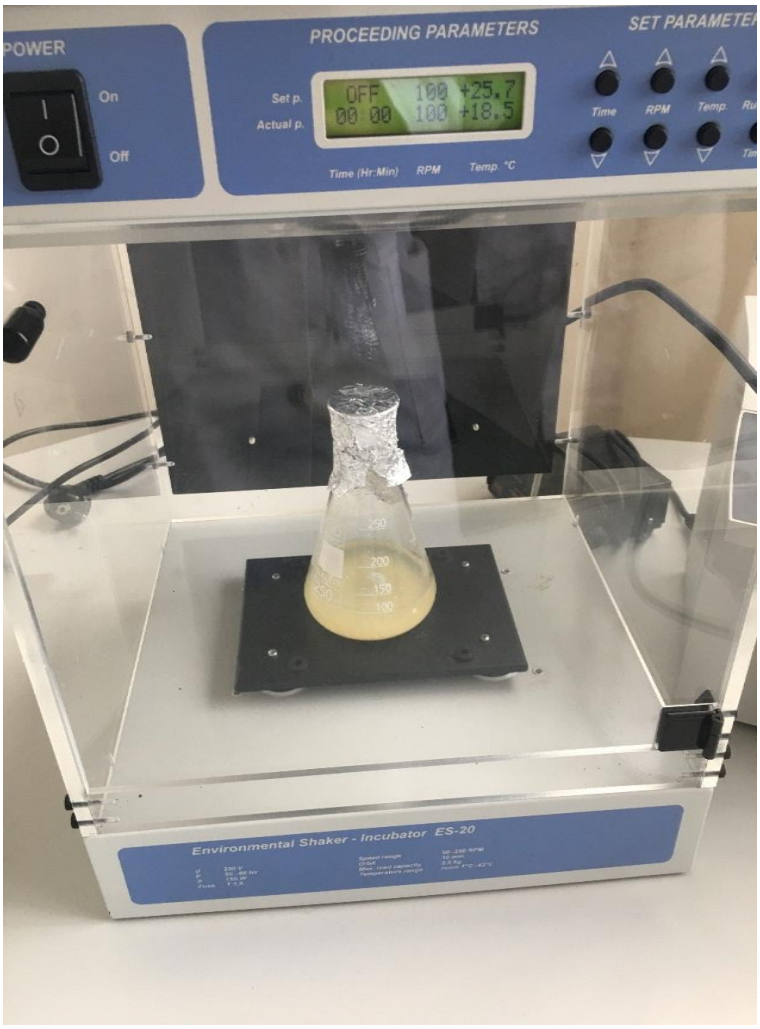
using a digital balance.

We fill the previous measurements in the Erlenmeyer, then we add 100 ml of distilled water.

Then we heat and mix at the same time using a magnetic hot plate stirrer for 15 mins (to obtain perfect mixing during the reaction which will increase our reaction rate).



Further, we will need to wait for 30 mins in order to cool down the mixture.

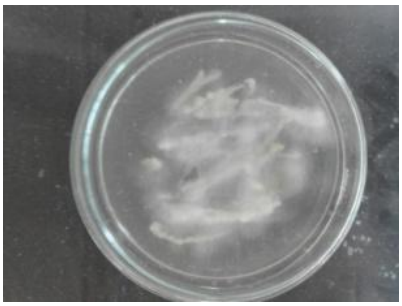


After cooling, we add a portion of the colony. Then we put the mixture on shaker for 7 days.

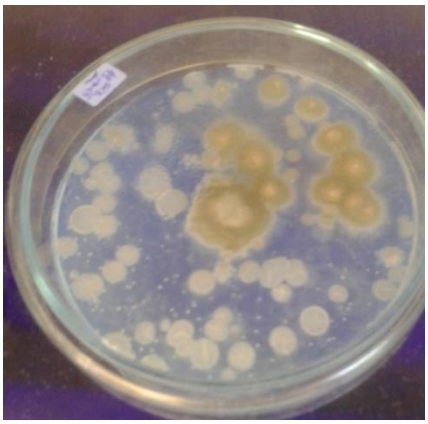
4 Results of experimental lab scale production of penicillin

4.1 From experiment 1

The bacteria of yogurt is living. This means that penicillin preparation is incorrect or incomplete



4.2 From experiment 2: preparation of penicillium colony



4.3 From experiment 3: Preparation of penicillin crystal by amino :

We get after the incubation in the fridge a few weeks of penicillin crystals

Saturday, June 30, 2018 1:28 PM long time incubation in the refrigerant: After filtration obtaining penicillin crystals 	

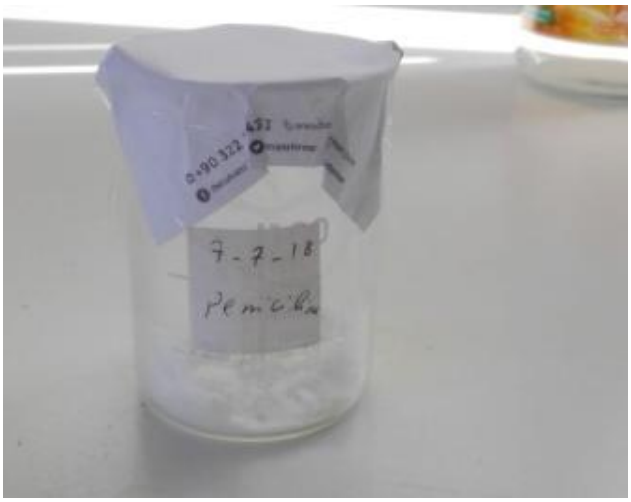
4.4 Experiment 4 preparation of ethyl acetate

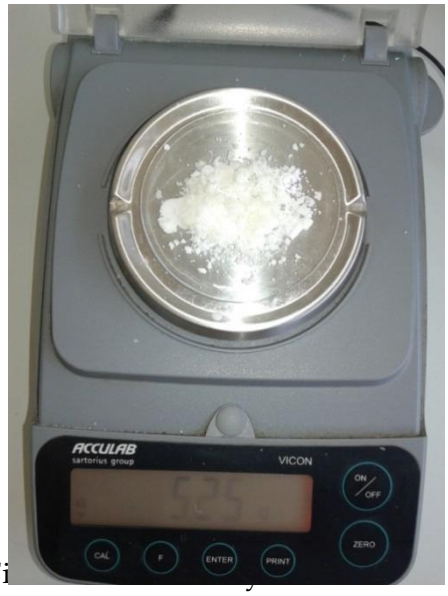
	we have a small percentage of acetic acid

4.5 Experiment 5: Preparation of ethyl acetate with the spirit of vinegar

	We have a high percentage of acetic acid

4.6 Experiment 6: preparation of liquid medium with peptone

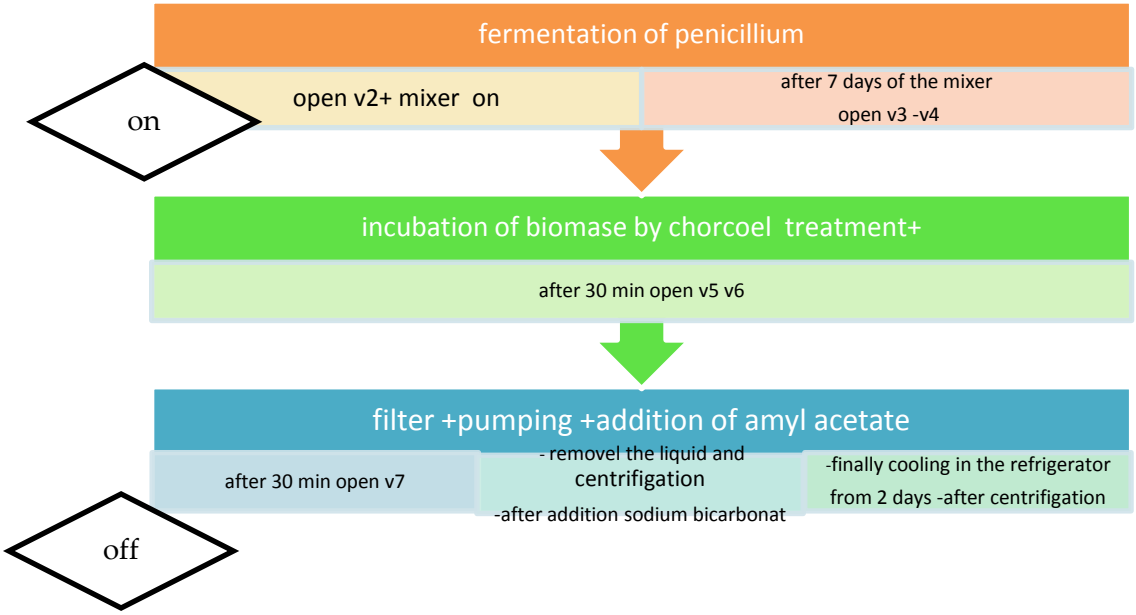




Fi

We obtain the 5.25 g of penicillin powder

5 Program (Flow Diagram) for Automatic Synthesis of penicillin in machine



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		

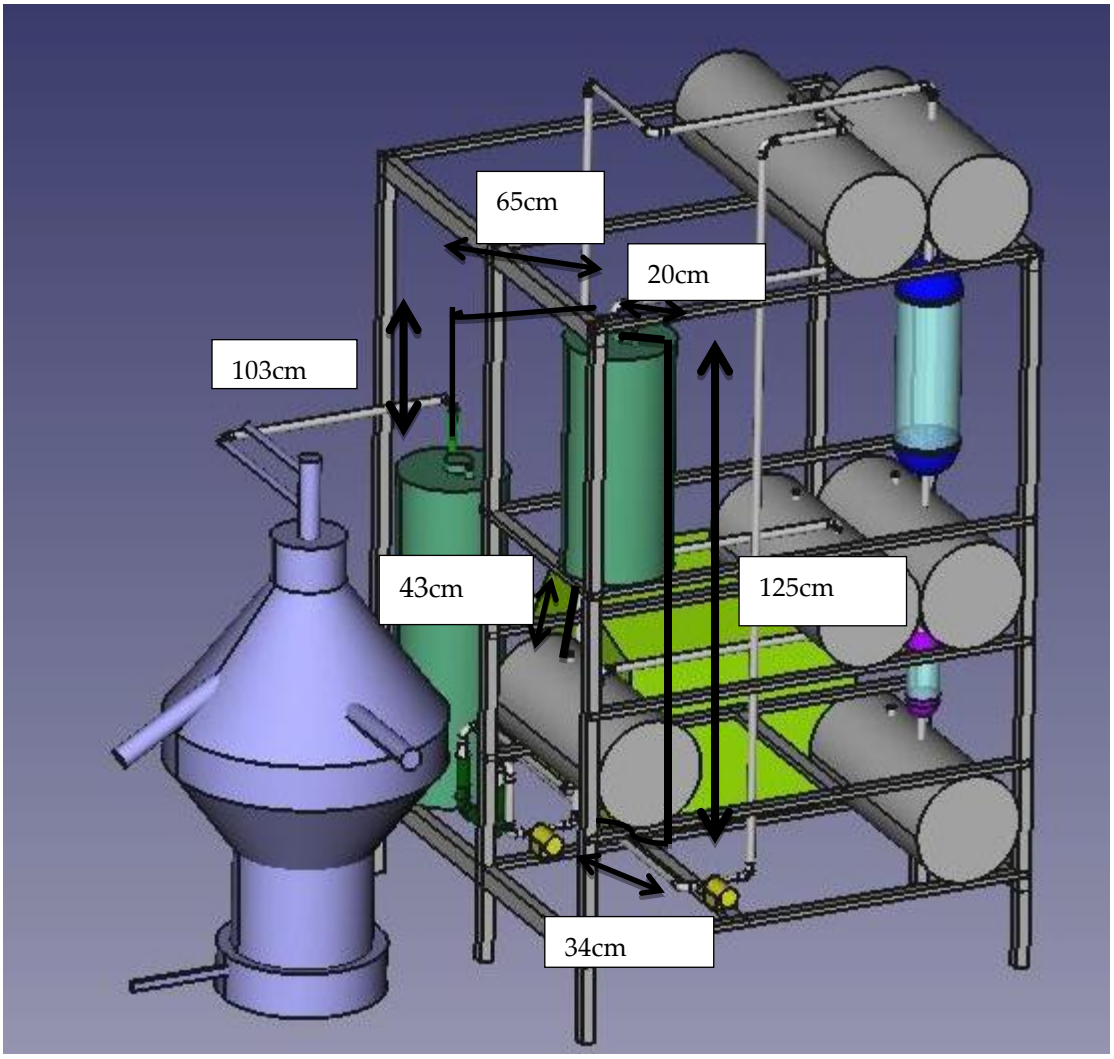
5.1 Installation issues concerning automation system (MEGI-APP)

date		task	المنفذ
2018-8-30	من 8 لل1	تشغيل ال mixerعلى الكهرباء	عبد الرحمن او محمد
	ساعتان	قص حديدة الmixer لتلائم الbioreacteur penicilline	
2018-8-29	نص نهار	تركيب فلتر الهواء	عبد الله
2018-9-2		تركيب ال الحنفيات و valves التحقق من ال5n 4n 3n 2n valves الautomatic	فاطمة عبد الرحمن
2018-9-2		تسكير الbioreacteur الفتحات	فاطمة

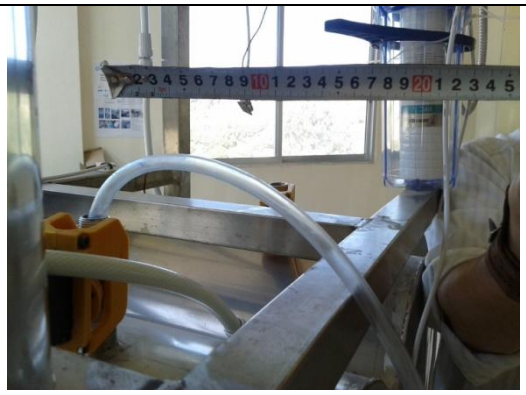
6 Installing Heat Sterilization Unit for the MEGBI-APP test rig

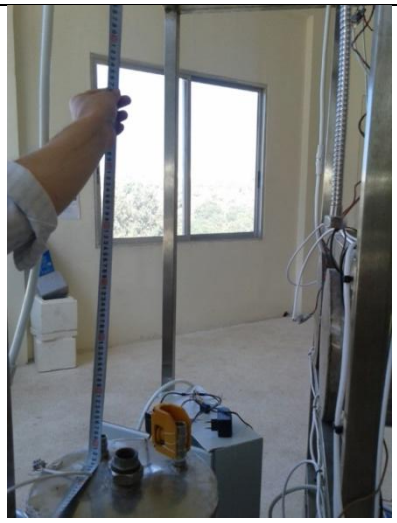
We will put an azone system to heat the water inside the bioreactor and control the 2-barrel test.

A system of tubes connects all the bioreactors use in our manipa to become sterilize the hot water vapor haudes so at the end when opening valve



New additions to the painting in black






Boiler for steam generation for autoclaving

7 الخطوات التالية (Next steps)

- The production of penicillin can suite by thin-layer chromotography to reveal their presence and by ELISA test kit to determine the concentration of penicillin.
- Treatments for penicillin aim to get semisynthetic penicillins like ampicillin.
- Synthese of penicilline with larger quantity.

8 References

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2. http://chemi101.blogspot.com/2013/01/blog-post.html
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4. derangedphysiology.com/main/core-topics-intensive-care/arterial-blood-gas-interpretation/Chapter 2.0.5/principles-po2-measurement-clark-electrode
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10. https://study.com/academy/lesson/how-does-penicillin-work-discovery-mechanism-properties.html