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Master I Applied Microbiology
Course: Industrial Microbiology (IM)

Lab. 1 Culture media preparation

Introduction

Microorganisms exhibit diverse nutritional requirements that are closely linked to their trophic groups, which categorize them based on their energy and carbon sources. Autotrophs, such as certain bacteria, synthesize their own food using inorganic materials and energy from sunlight (photoautotrophs) or chemical reactions (chemoautotrophs).

Heterotrophs, including most fungi and many bacteria, rely on organic compounds for both carbon and energy, breaking down complex substances through metabolic processes.

Microbial culture media are formulated to provide the necessary nutrients for the growth and maintenance of microorganisms in a laboratory setting. The composition typically includes sources of carbon, nitrogen, vitamins, minerals, and water. Media can be classified into several types based on their properties and purposes: simple media, enriched media, selective media, and differential media...

In industrial bioprocessing, microbial culture media are carefully designed to optimize the growth and productivity of microorganisms for applications such as fermentation and bioproduction. The composition of these media typically includes a balance of carbon sources (like glucose or molasses), nitrogen sources (such as yeast extract or ammonium salts), vitamins, minerals, and sometimes specific growth factors tailored to the target organism. Common types of media used in industrial settings include **rich media**, which support high cell densities and product yields; **minimal media**, formulated for cost-efficiency when growing organisms that can thrive on simple nutrient sources; and **optimized media**, which are customized through metabolic engineering to enhance specific metabolic pathways. Selecting the appropriate media type is crucial for maximizing the efficiency and economic viability of microbial processes in industries like pharmaceuticals, food production, and biofuels.

Objective

The objective of this lab session is to learn how to prepare and sterilize different liquid and solid culture media.

Material

- Beef extract, Yeast extract
- Tryptic peptone
- Glucose, NaCl, K_2HPO_4, KH_2PO_4, $MgSO_4$ $6H_2O$, NH_4Cl
- Agar-agar
- Skimmed milk powder
- Distilled water
- NaOH, HCl
- Erlenmeyers (250ml), screw-capped glass tubes, beakers, glass bottles
- Graduated cylinder (500 ml), graduated pipettes
- Petri dishes
- Balance
- pH meter
- Magnetic heating plate
- Cotton, gauze, aluminum
- Autoclave.

Experimental protocol

- Weigh the constituents necessary to prepare 500 ml of each medium as indicated in table 1 and 2.
- In a 1L beaker, put 350 ml of distilled water, add the previously weighed constituents and homogenize after each addition. Use a magnetic stirrer in this step. Heat if necessary (in the case of solid media).
- Adjust the pH of the media with NaOH or HCl.
- Make up the volume to 500 ml with distilled water, homogenize.
- Distribute the nutrient broth (NB) into glass tubes at a volume of 10 ml per tube using a graduated pipette; close the caps without tightening them completely, dispense the remaining medium in Erlenmeyer flasks and close them tightly with cotton covered with gauze, further cover with aluminum foil.
- Dispense the solid media and the basal medium II (BMII) in glass bottles or flasks, close or cover.
- Autoclave at 121°C for 15 minutes for nutrient agar (NA) and NB and 10 minutes for MNA and BMII .
- Allow the solid media to cool to a temperature of around 48°C, pour into Petri dishes.
- Allow the agar to solidify then invert the plates and incubate them at 37°C.

Table 1. Composition of culture media (NB, NA and MNA)

Constituents (g/L)	Culture media			pH
	Nutrient broth (NB)	Nutrient agar (NA)	Milk nutrient agar (MNA)	
Beef extract	3	3	3	7.3± 0.2
Peptone	5	5	5	7.3± 0.2
Sodium chloride	5	5	5	7.3± 0.2
Agar-agar	-	15	15	7.3± 0.2
Skimmed milk powder	-	-	10	7.3± 0.2

Table 2. Composition of basal medium II (BMII) (pH7.0)

Component	Quantity (g/L)
Glucose	1
NH_4Cl	0.5
NaCl	0.5
K_2HPO_4	0.3
KH_2PO_4	0.4
$MgCl_2\ 6H_2O$	0.1
Yeast extract	0.1

Questions:

What is the importance of each step ?

Why do culture media need to be sterilized before use? Why 10 min autoclaving for MNA and BMII?

Why do we have to invert the Petri dishes after the agar solidifies?

Why should we let the solid medium cool to 48°C before pouring it into the Petri dishes?

What is the source of carbon in the media of the tables above?

Indicate if your agar plates appear sterile after 24 hours of incubation.

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