# THE EFFECT OF LAURIC ACID AS ANTIMICROBIAL AGENT IN CHITOSAN-BASED PACKAGING FILM


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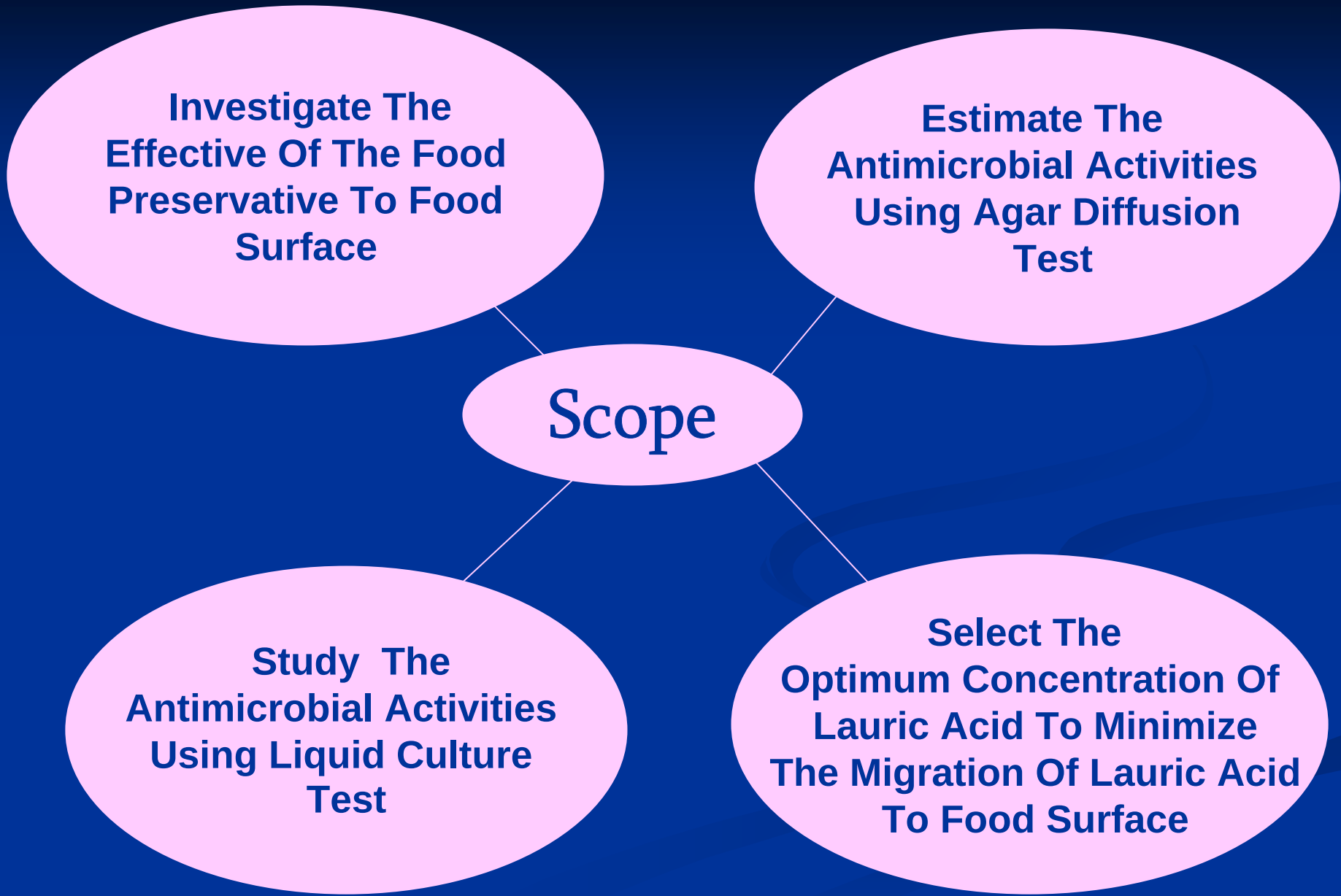
# BACKGROUND

# OBJECTIVE

To study the effect of Lauric acid as antimicrobial agent in Chitosan -based packaging film.


Scope
Investigate The Effective Of The Food Preservative To Food Surface
Estimate The Antimicrobial Activities Using Agar Diffusion Test
Study The Antimicrobial Activities Using Liquid Culture Test
Select The Optimum Concentration Of Lauric Acid To Minimize The Migration Of Lauric Acid To Food Surface

# INTRODUCTION

# Active Packaging

- As a barrier between the product and the outside environment in that interactions between the food and package occur that ultimately improve product quality and safety (Alvarez, 2000)

- It can control, and even react to, events taking place inside the package

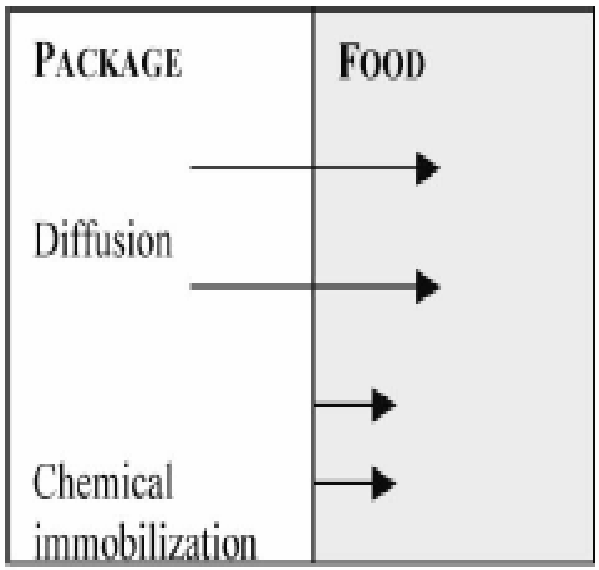
| Active Packaging System | Application |
| --- | --- |
| Oxygen scavenging | ~Most food classes |
| Carbon dioxide production | ~Most food affected by moulds |
| Water vapour removal | ~Dried and meld-sensitive foods |
| Ethylene removal | ~Horticultural produce |
| Ethanol release | ~Baked foods (where permitted) |
| Antimicrobial Activity | ~Most food classes |

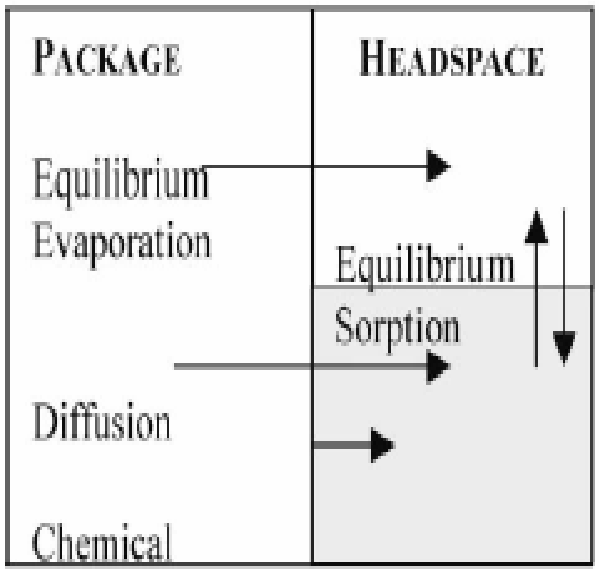
# Antimicrobial Packaging

- To extend the lag period and reduce the growth rate of microorganisms to prolong shelf life and maintain food safety

- To reduce microbial growth of nonsterile foods or maintain the stability of pasteurized foods without post-contamination

# Antimicrobial Packaging System



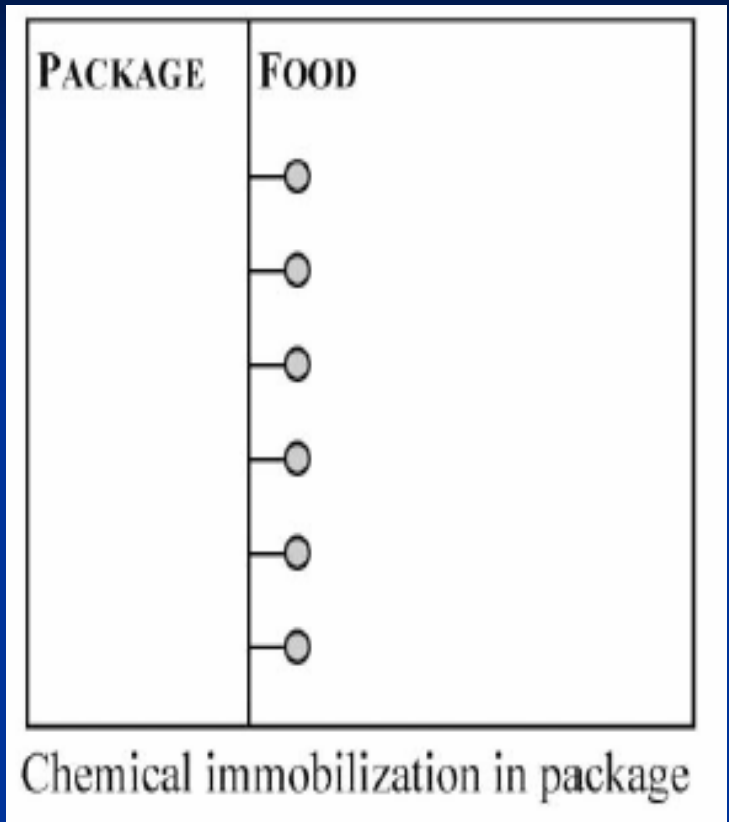

- If the void volume of solid food products is assumed as a kind of headspace, most food packaging systems represent either a package/food system or a package/headspace/ food system

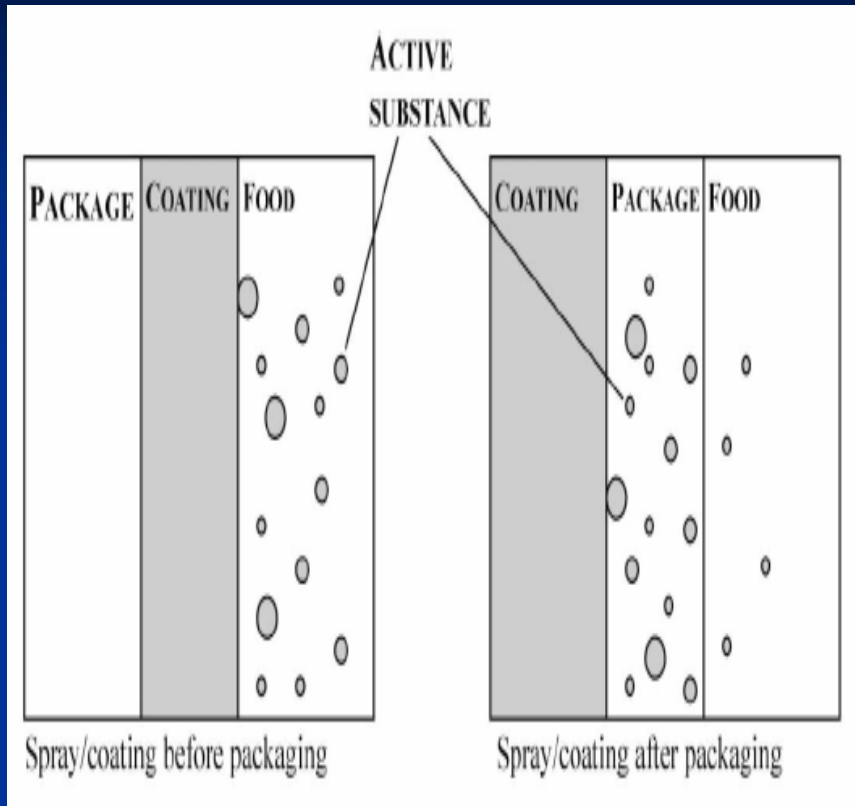
- Diffusion between the packaging material and the food and partitioning at the interface are the main migration phenomena involved in this system.

- Antimicrobial agents may be incorporated into the packaging materials initially and migrate into the food through diffusion and partitioning.

# Type Of Antimicrobial Packaging

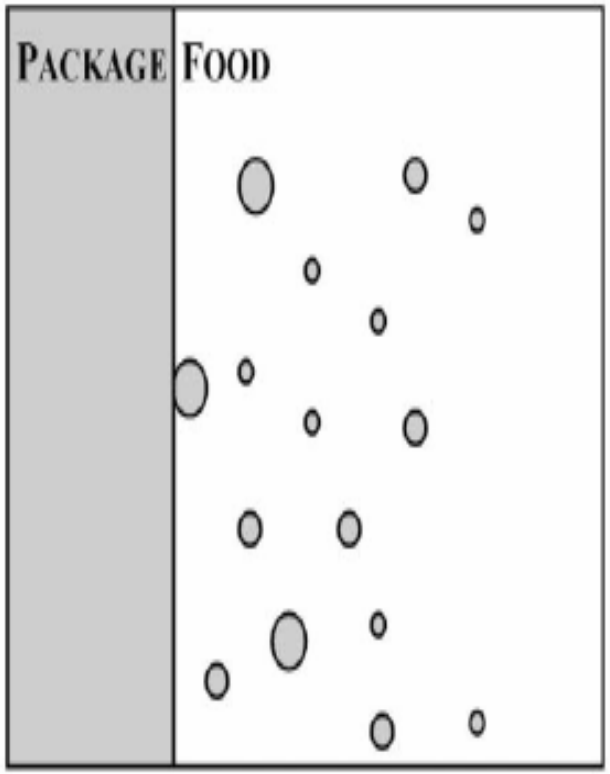
- Immobilization of antimicrobials to polymers by ion or covalent linkages
- Coating or adsorbing antimicrobials onto polymer surfaces
- Incorporation antimicrobial agents directly into polymers




**Immobilization of antimicrobial agent in package**

**Coating antimicrobial agent in package**



Incorporation in package

✓In the mechanism incorporate antimicrobial agent, antimicrobial films might allow for migration of the antimicrobial to the film surface , therefore a continued antimicrobial effect at the food surface during extended exposure.

✓Direct addition of antimicrobials to food will result in an immediate reduction of bacterial population.

# Incorporation Antimicrobial Agents

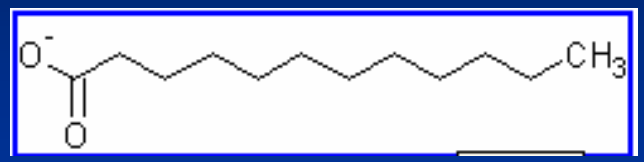
- Films with low diffusion rates were desirable since they maintained higher surface concentrations for longer periods
- The gradual release of an antimicrobial from a packaging film to the food surface may have an advantage over dipping and spraying
- Antimicrobial activity may be rapidly lost due to inactivation of the antimicrobials by food components or dilution below active concentration due to migration into the bulk food matrix.

# Factors To Be Considered in the Manufacturing of Antimicrobial Films

- Casting process conditions and residual antimicrobial activity
- Characteristics of antimicrobial substances and foods.
- Storage temperature
- Chemical interaction of additives with film matrix
- Physical properties of packaging materials
- Mass transfer coefficients and modeling

# Antimicrobial Agent

## Lauric acid

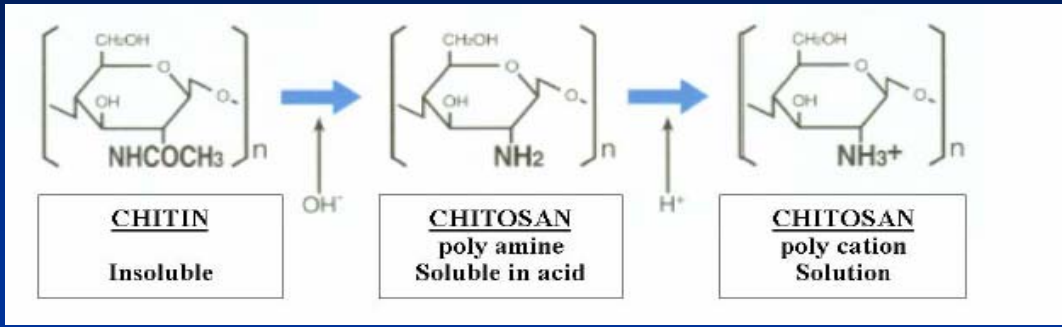



- ✓ Extract of coconut oils
- ✓ Is a medium-chain fatty acid
- ✓ Function : Antiviral, antibacterial and antiprotozoal
- ✓ Additional Beneficial : Formed into monolaurin in the human or animal body

According to the published research, Lauric Acid is one of the best "inactivating" fatty acids, and its monoglyceride is even more effective than the fatty acid alone (Kabara, 1978; Sands et al., 1978; Fletcher et al., 1985; Kabara, 1985).

# Film Based

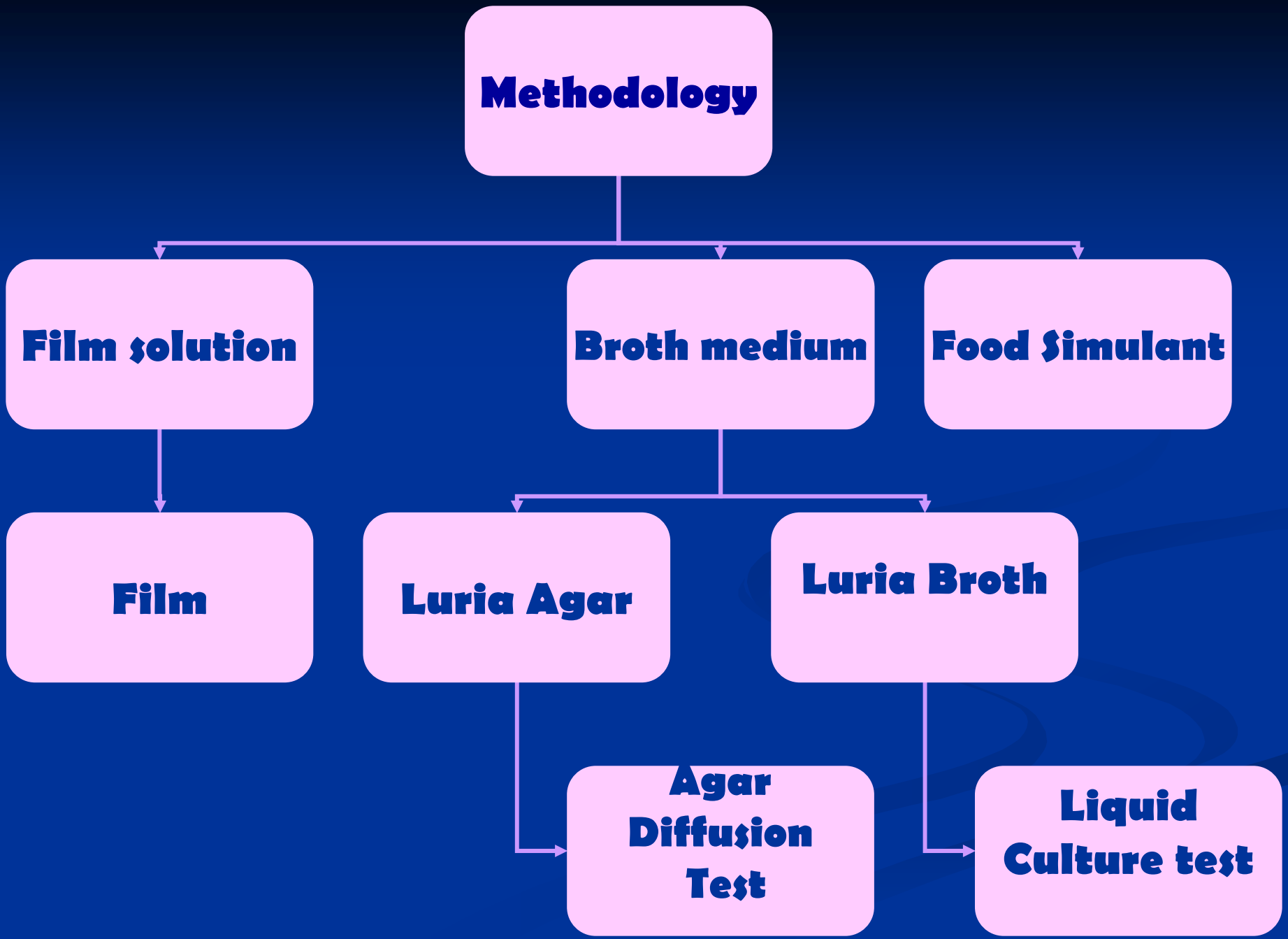
## Chitosan




- Insoluble in water
- Soluble in acidic solvent below pH 6.
- Organic acid are used for dissolving chitosan
- Chitosan solution's stability is poor above about pH 7

- Most commercially available chitosan preparations are more than 85 % deacetylated of chitin, and have molecular weights between 100kDa and 1000kDa.
- They are usually complexed with acids, such as acetic or lactic acids.
- An important property of chitosan is its positive charge in acidic solution.
- This is due to the presence of primary amines on the molecule that bind protons according to the equation.

# MATERIAL & METHODOLOGY


Methodology
Film solution
Broth medium
Food Simulant
Film
Luria Agar
Luria Broth
Agar Diffusion Test
Liquid Culture test

## Raw material

- Chitosan powder
- Lauric acid
- Glycerin
- Acetic Acid 1 %
- Luria Broth
- Luria Agar
- Food Simulant

## Equipment

- Petri disc
- Homogenizer
- Universal Bottles
- Incubator Shaker
- Spectrophotometer
- Incubator
- HPLC

# Media- LB Broth

- In a bottle with a cap, dissolve 2.5 grams of Luria broth powder in 100 ml of distilled water.
- Cap the bottle and autoclave for 30 minutes.
- Refrigerate to prevent contamination.


# Media-LB Agar Plates

## Step 1

- To make 4 to 5 plates of Luria broth agar:
- Place 2.5 grams of LB powder and 1.5 grams of agar in 100 ml of distilled water.


# Media-LB Agar Plates

## Step 2

- Autoclave until the solution is clear.

# Media-LB Agar Plates

## Step 3

- Allow the LB agar solution to cool until you can just hold the bottle with your bare hand.
- Pour the LB agar solution into 2 petri dishes and cover with the laminar flow.

# Media-LB Agar Plates

## Step 4

- Let stand 30 minutes to dry and then keep it into refrigerator.

# Food Simulant

| Food Type | Food Simulant | Abbreviation |
|---|---|---|
| Aqueous Foods ( pH > 5 ) | Distilled water | Simulant A |
| Acidic foods (pH <4,5) | Acetic acid 3 % | Simulant B |
| Alcoholic foods | Ethanol 10 % (v/v) | Simulant C |
| Fatty foods | olive oil | Simulant D |

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# RESULT & DISCUSSION

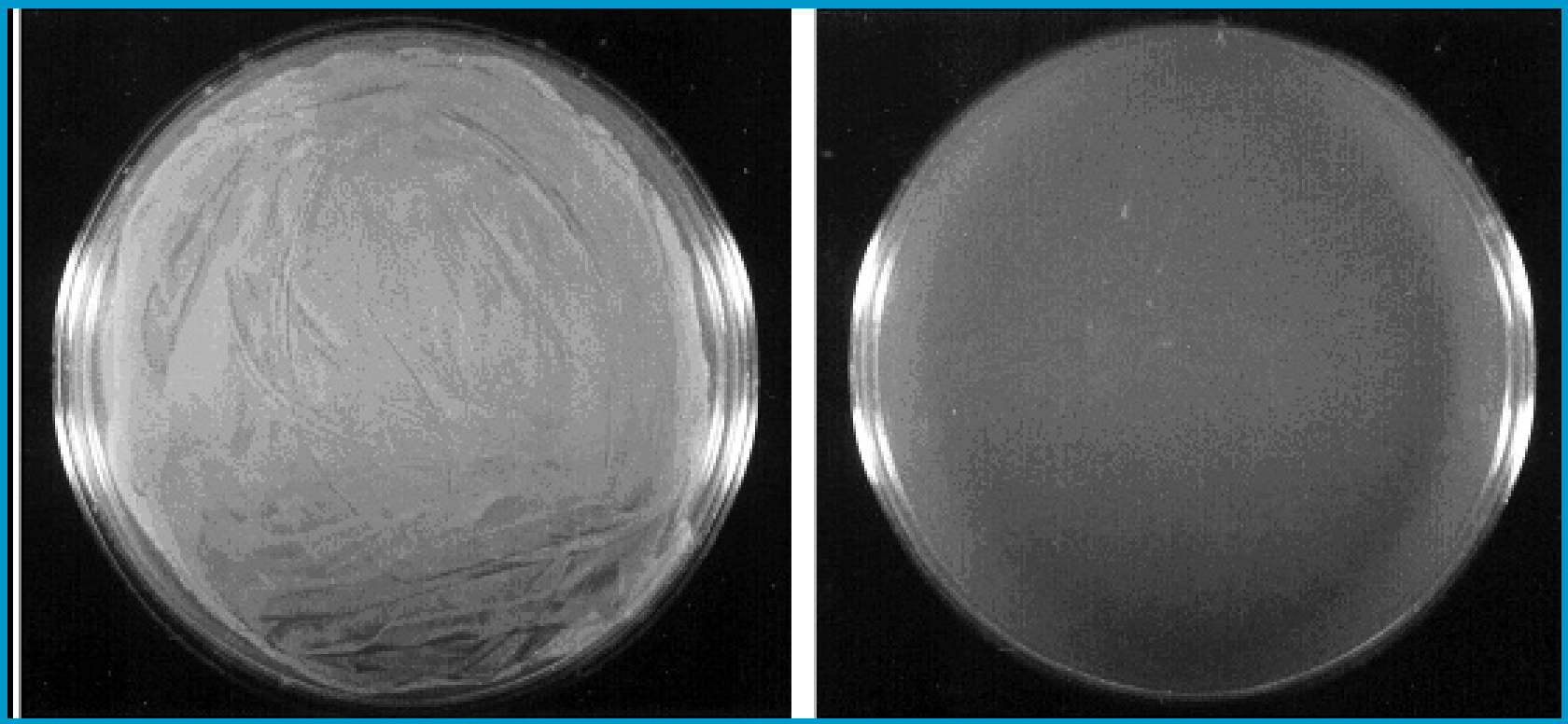
# Film Formulation

- Best formulation :
- Chitosan powder = 2g/100ml water
- Adding plasticizer Glycerin
  = 2 mL
- Acetic acid 1% as dilution for chitosan
- Casting volume = 20ml / Petri dish

# Film Appearance

- Yellow Transparent, homogenous, strength, less elastics
- Concentration Lauric acid increase, film quality decrease

# Lawns of *E. coli* incubated at 37°C and 4°C



37°C

4°C

# AGAR DIFFUSION TEST

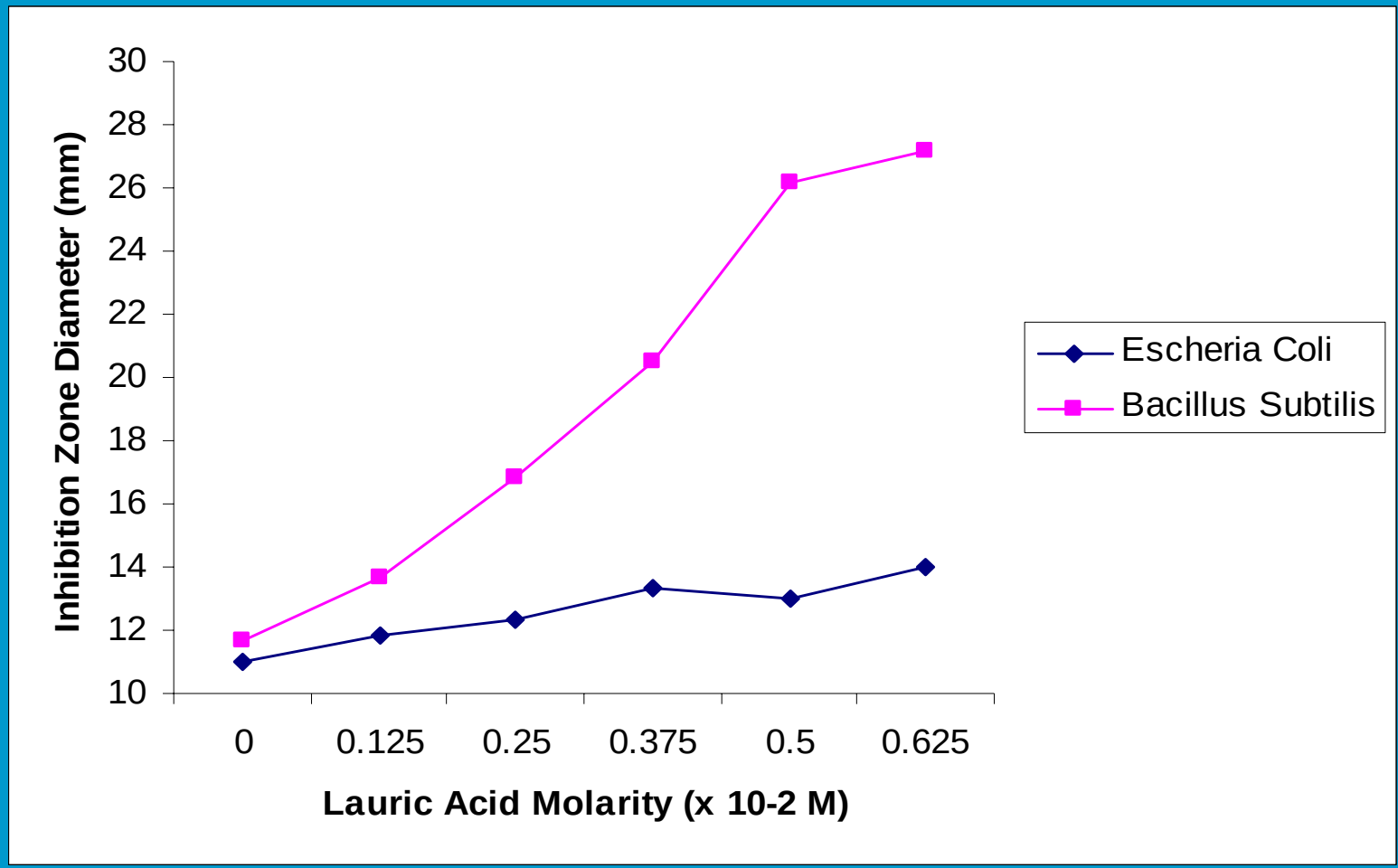
B.Subtilis 0.000 M

B.Subtilis 0.625 x 10-2 M

E.Coli 0.000 M

E.Coli 0.375 x 10-2 M

# Comparison Inhibition Zone Between E.Coli and B.Subtilis

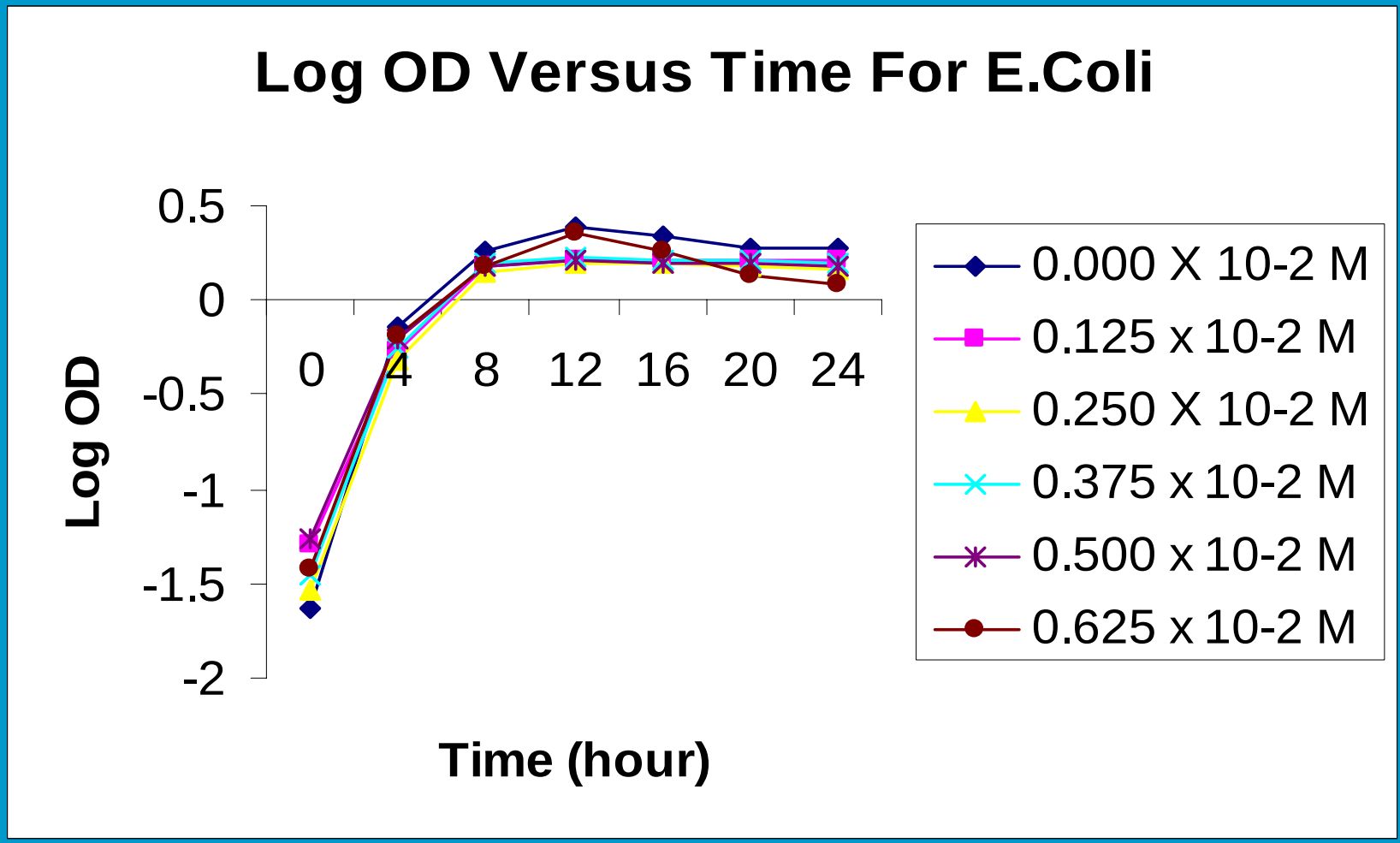


# Relation Between Inhibition Diameter with Concentration Of Lauric Acid

- Lauric acid had been used as the antimicrobial compound
- For E.Coli, it shows small impact on the inhibition growth after 24 hour of incubation
- For B.Subtilis, it shows that the concentration of Lauric acid in film increased, the inhibition area also increased . At highest concentration Lauric acid, the diameter become almost 3 times from

# Liquid Culture Test

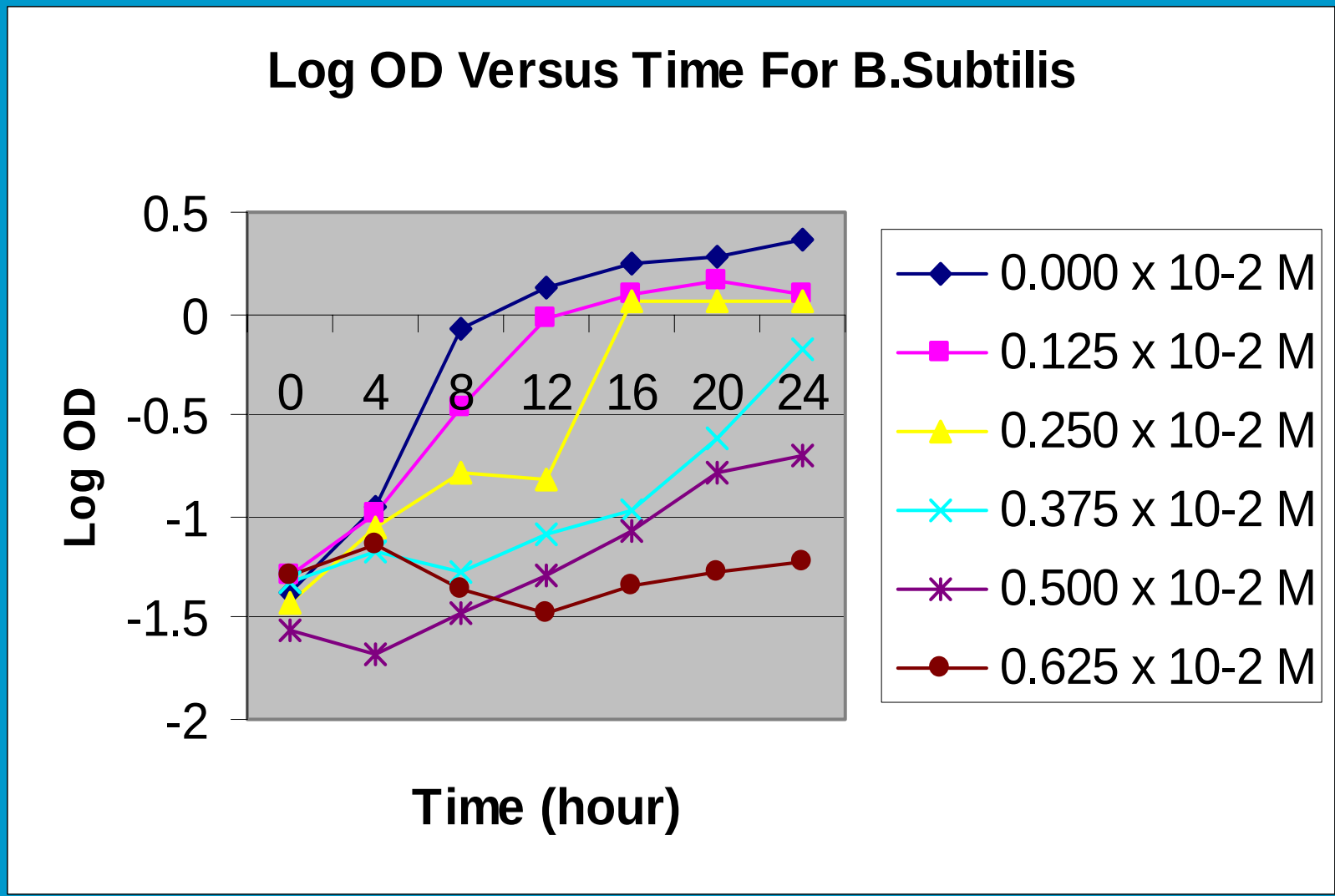
# Growth curve for *E.coli*



# Liquid Culture Test For E.Coli

- The growth curve are similar pattern for all different concentration of lauric acid used
- Growth of E.Coli unaffected by present of Lauric acid
- Deceleration phase at 10hours
- E.Coli has an outer membrane supported by a thin peptidoglycan layer

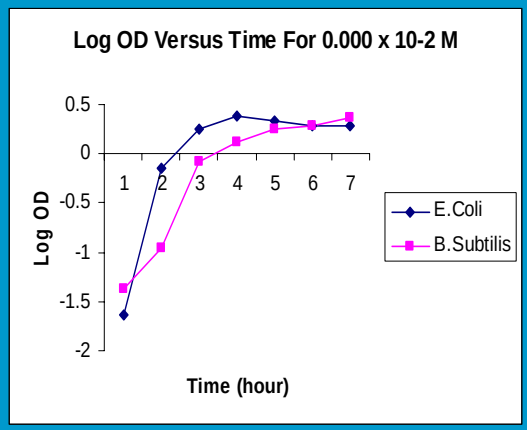
# Growth Curve for *B.Subtilis*

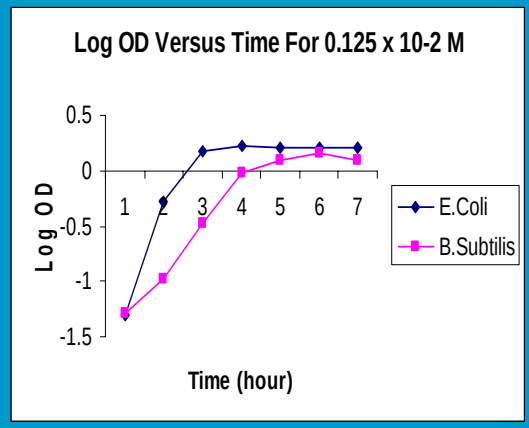


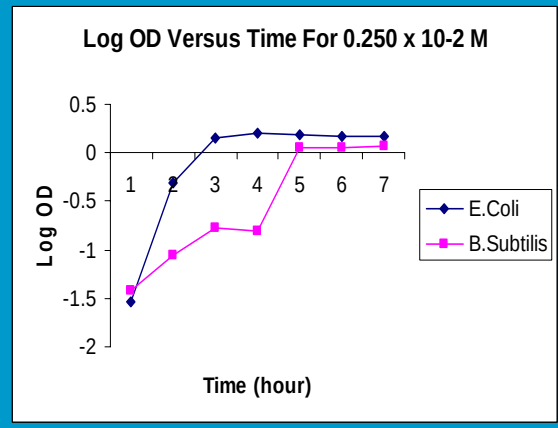
# Liquid Culture Test For B.Subtilis

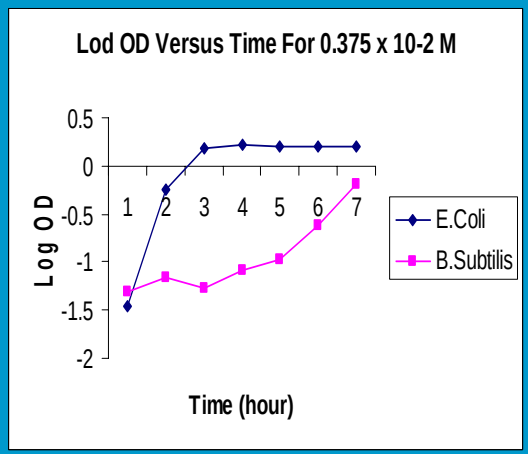
- Selected $0.625 \times 10^{-2}$M Lauric acid because it delay the lag phase for 12 hours
- Gram positive cell do not have outer membrane
- The structure of B.Subtilis easily to be destroyed or affected by lauric acid

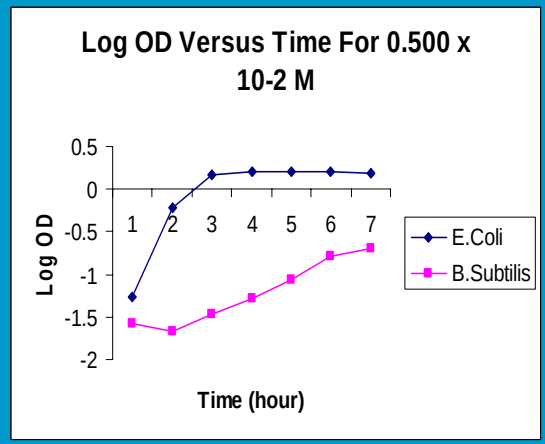
# Comparison Growth Curve Of Both Bacteria

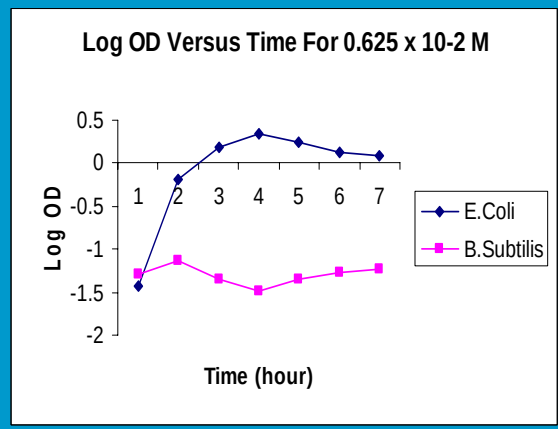


# CONCLUSION

- ✓ Lauric acid has a good potential as antimicrobial agent
- ✓ Increasing of lauric acid concentration give small impact on E.Coli inhibition zone
- ✓ For B.Subtilis, clear zone diameter expand by increasing lauric acid concentration
- ✓ Lauric acid inhibit against gram positive microorganism
- ✓ Lauric acid  did not change the growth curve pattern of E.Coli
- ✓ Based on B.Subtilis longest lag phase time, the most optimum concentration of Lauric acid is 0.625 x 10-2 M.

# RECOMMENDATION

- For Lauric acid, a more suitable formulation is needed to get a smoother and higher quality film
- Further study can be carried out the physical and mechanical properties of the film such as thickness, strength, tensile and melting point of film
- Varies the use of bacteria and extend the time of incubation

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# THANK YOU