

FE 305 Experiment 5

Bacteriological Examination of Bottled Soft Drinks and Fruit Juice

Contents

- Total Aerobic Mesophilic Bacteria Count
- Mold and Yeast Count

Experiment No from Book: **37-45**

Samples needed for this experiment

- Bottled Coke
- Fresh squeezed Fruit Juice
- Pasteurized Fruit Juice

Purpose and Importance

- Fruit Juice can be defined as juice portion of the fruit without any addition of sugar or water.
- Fruit concentrate is obtained by removing excess water from juice for storage and later addition.
- Fruit nectar is obtained by concentrate and added sugar.
- Fruit mix is the mixture of some natural aroma of fruits , water , and sugar.
- Bottled soft drinks are non alcoholic beverages.



Purpose and Importance

- Mold and Yeast growth is the main spoilage factor at Fruit Juice and Bottled Soft drinks because they have high acid and sugar content which prevents most of the bacteria growth.
- In order to prevent this, some additives and preservatives may be added into fruit juice.

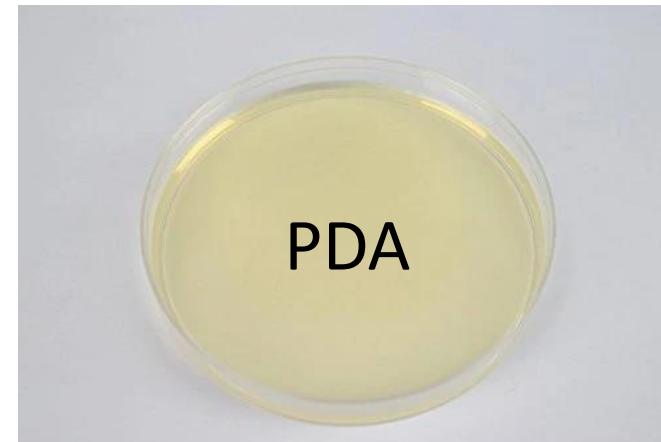


Tests and Analysis

- Total Count
 - Media: PCA
 - Method: Spread Plate
 - Incubation: 37 C for 24 hours



- Mold and Yeast Count
 - Media: PDA
 - Method: Spread Plate
 - Incubation: 25 C for 3-5 days

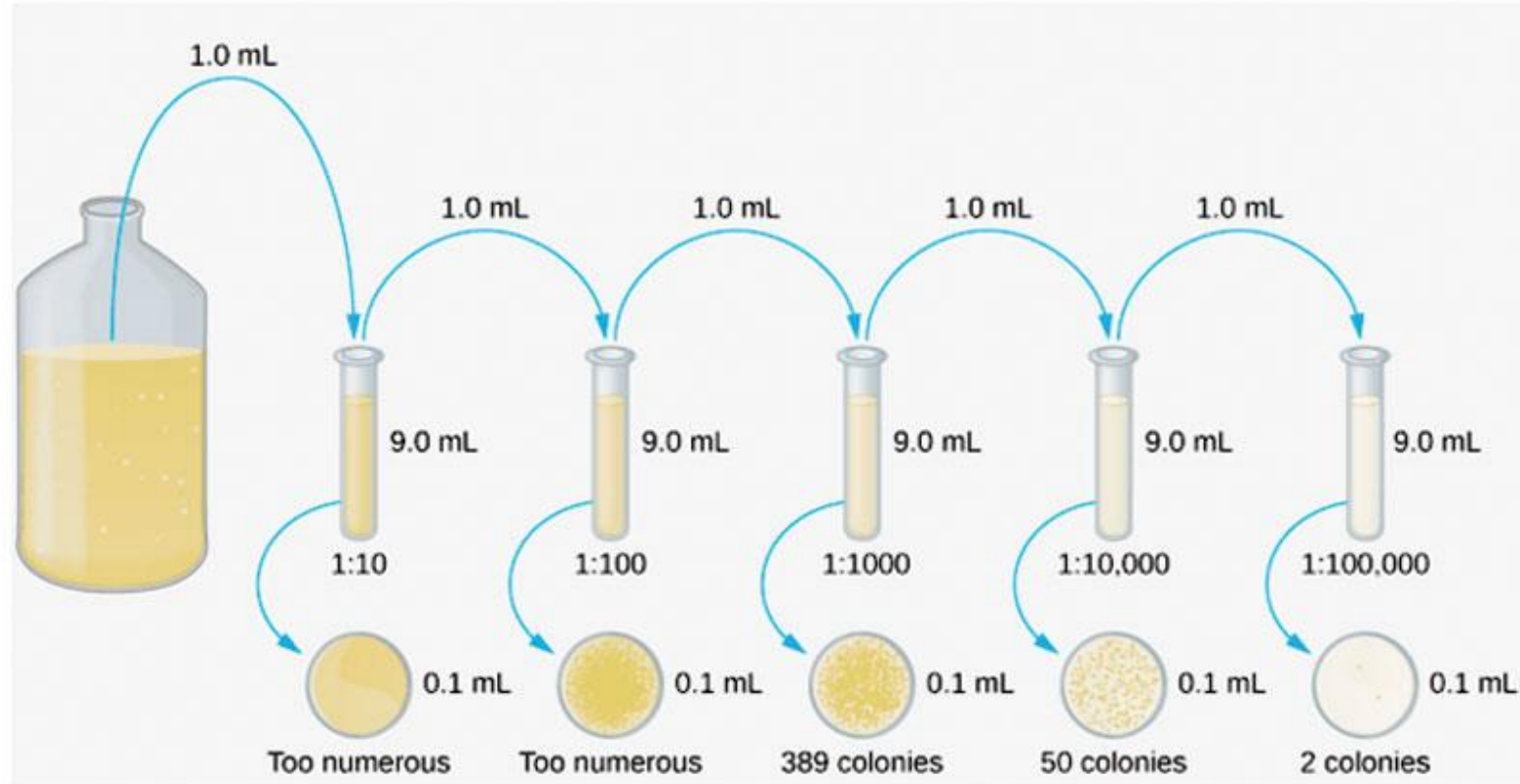


Material and Methods

- Fresh Fruit juice, Pasteurized Fruit juice and coke samples prepared earlier as 10^{-1} diluted sample and dilutions.
- Spreader
- Sterile pipette
- Incubator
- Alcohol
- Bunsen burner
- Sterile PCA, and PDA

Procedure

- Take 0.1 ml of sample from each dilution by using sterile pipette at Aseptic Conditions.
- Place the sample on PCA and PDA petri plates near flame.



10^{-1}

10^{-2}

10^{-3}

10^{-4}

10^{-5}

Total Count

PCA

PCA

PCA

PCA

PCA

Mold and Yeast

PDA

PDA

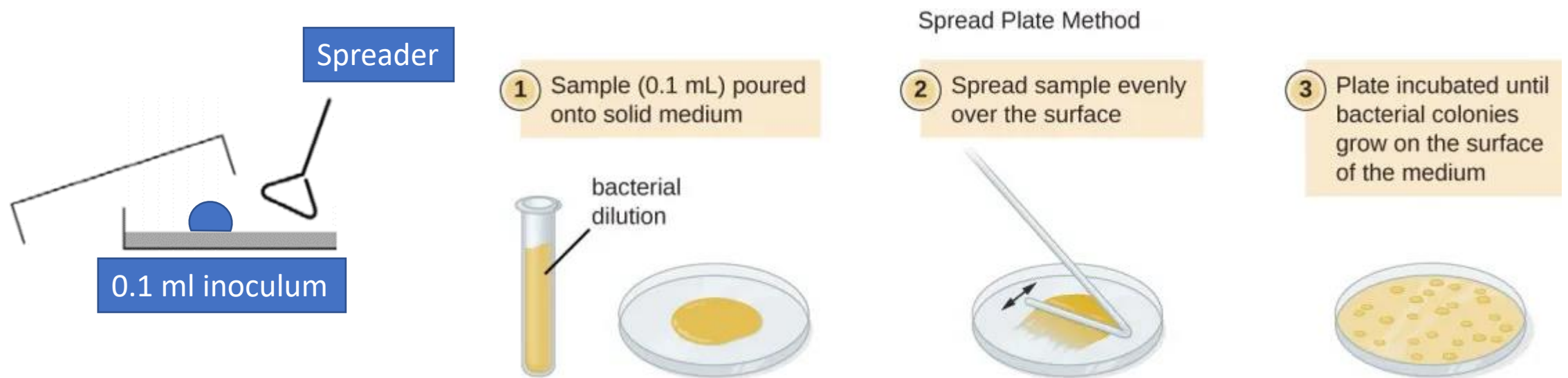
PDA

PDA

PDA

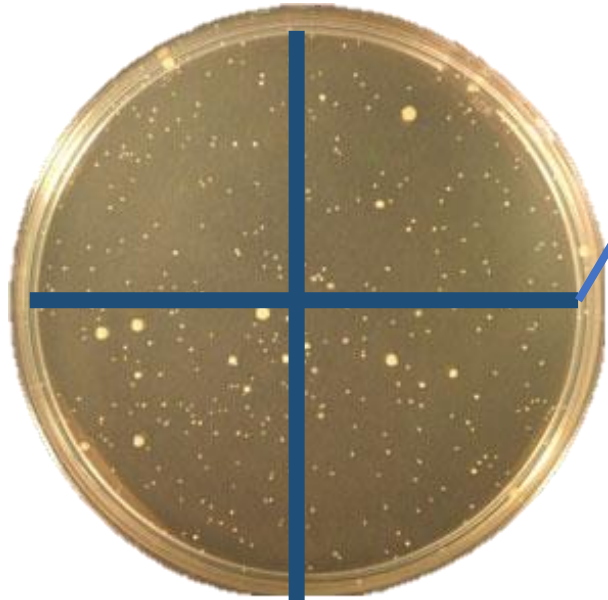
Procedure

- Sterilize spreader with dipping in alcohol and passing through flame.
- Spread the sample on petri plate by using spreader.



Results

- Count formed colonies on every petri plate for each dilution and record results on the given table below.



- You can divide petri into equal parts to count easily.
- Ex: For Home made yogurt
 - Non → Too nuber to count → TNTC
 - -1 → 546
 - -2 → 186
 - -3 → 34
 - -4 → 0

| Sample names | Dilutions | | | | | |
|-------------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| | non | 10^{-1} | 10^{-2} | 10^{-3} | 10^{-4} | 10^{-5} |
| Fresh Fruit Juice | TNTC | 546 | 186 | 34 | 0 | 0 |
| Pasteurized Fruit Juice | | | | | | |
| Coke | | | | | | |

Calculation

- # of microorganisms / ml of juice = $\frac{\text{Count of microorganisms in one petri}}{\text{inoculum amount in one petri}} \times \text{Dilution factor}$
- Ex: For Fresh Fruit Juice take only counts between $30 < x < 300$ colonies into calculation.

- Non: TNTC

- -1: 546 → No calculation above 300

- -2: 186

- -3: 34

- -4: 0 → No calculation below 30

$$\frac{186}{0.1 \text{ ml}} \times \frac{1}{10^{-2}}$$

$$\frac{34}{0.1 \text{ ml}} \times \frac{1}{10^{-3}}$$

- Take average of these two results

- # of microorganisms / g or ml of water = $\frac{186000 + 340000}{2} = 263000$ microorganism / ml of water

Same Calculation for Mold and Yeast count

Evaluation of Results

- **Total count** → Calculate and record # of microorganisms / ml of juice
- **Mold and Yeast count** → Calculate and record # of molds / ml of juice

Total count gives information about general microbial conditions of yogurt.

Mold and Yeast count is important for spoilage and shelf life of Fruit Juice.