ADARSH COLLEGE

OMERGA, DIST. OSMANABAD

DEPARTMENT OF MICROBIOLOGY

CERTIFICATE

Practical paper – IV Industrial Food and Dairy Microbiology

Certified that Shri / Miss____

has satisfactorily completed the course of practical work in

M. Sc. I YEAR (SEMESTER I) prescribed by Dr. B. A. M. University,

Aurangabad under my supervision in the Microbiology Laboratory

during the academic year 20 – 20

Staff member incharge

Date:

Head of the department

Date:

Signature of the Examiner

Date:

Exam Seat No. _________________________

Paper – **Industrial Food and Dairy Microbiology**

Expt. No. 01 Date

Production and Estimation of Lactic acid by Lactobacillus species

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Aim: To produce and estimate Lactic acid by Lactobacillus species

Theory:

Lactic acid, also known as milk acid, is a chemical compound that plays a role in various biochemical processes. Lactic acid is a carboxylic acid with the chemical formula$C_3H_6O_3$. It is classified as GRAS (generally regarded as safe) by Food and Drug Authority (FDA) in the USA and its annual consumption is estimated to be 30000 tonnes. Lactic acid is produced by many organisms. The most important lactic acid producing bacteria is Lactobacillus. Commercial lactic acid is produced naturally by fermentation of carbohydrates such as glucose, sucrose, or lactose.

1. It is used in the baking industry.
2. In medicine it is sometimes used to introduce calcium in to the body in the form of calcium lactate, in diseases of calcium deficiency.
3. Esters of lactic acid are also used in the food industry as emulsifiers.
4. Lactic acid is used in the manufacture of rye bread.
5. It is used in the manufacture of plastics.
6. Lactic acid is used as acidulant/ flavoring/ pH buffering agent or inhibitor of bacterial spoilage in a wide variety of processed foods. It has the advantage, in contrast to other food acids in having a mild acidic taste.
7. It is a very good preservative and pickling agent.
8. Lactic acid has many pharmaceutical and cosmetic applications and formulations in topical ointments, lotions, anti acne solutions, humectants, parenteral solutions and dialysis applications, for anti carries agent.
9. Its biodegradable polymer has medical applications as sutures, orthopaedic implants, controlled drug release, etc.

Requirements

1. Pure culture of *Lactobacillus bulgaricus* and *L. brevis*
2. Production medium

Water	100 ml
Peptone	1.5 gm
Meat extract	1.5 gm
Yeast extract	1.5 gm
Glucose	1gm
Sodium Acetate	0.3 gm
Ammonium Citrate	0.3 gm
$MgSO_4$	0.35 gm
$MnSO_4$	0.03 gm
pH	6.2 to 6.4

Sterilize at 110^0 C for 40 minutes

3. 0.1 N NaOH (400 mg NaOH in 100 ml water)
4. Phenolpthalein indicator

Procedure:

1. Sterilized production medium is inoculated with 10 ml of pure culture of *Lactobacillus bulgaricus* in none flask and *L. brevis* in another flask.
2. Then flasks are incubated at 37^0 C for 24 hours.
3. Then broth is centrifuged at 1000 RPM for 10 minutes.
4. Supernatant is collected and used for lactic acid estimation.
5. 5 ml of supernatant and 5 ml D/W is taken and boiled to drive of CO_2.
6. Then it is cooled and 2 to 3 drops of phenolpthalein indicator are added.
7. It is titrated against 0.1 N NaOH till the light pink colour develops.
8. Percentage of lactic acid is calculated by formula

$$\text{Lactic Acid Produced} = \frac{\text{Burette reading X Normality of NaOH X Mol. Wt of Lactic Acid}}{\text{Amount of sample taken X 10}}$$

Observation table

Culture	**Ml of NaOH Burette reading**	**% of Lactic acid**
L. bulgaricus		
L. brevis		

Calculation:

$$\text{\% of Lactic Acid By } \textit{L. bulgaricus} = \frac{\text{Burette reading X Normality of NaOH X Mol. Wt of Lactic Acid}}{\text{Amount of sample taken X 10}}$$

$$= \frac{BR \times 0.1 \times 90}{4 \times 10}$$

$$\text{\% of Lactic Acid By } \textit{L. brevis} = \frac{\text{Burette reading X Normality of NaOH X Mol. Wt of Lactic Acid}}{\text{Amount of sample taken X 10}}$$

$$= \frac{BR \times 0.1 \times 90}{4 \times 10}$$

Result:

1. Amount of lactic acid produced by *L. bulgaricus* is ___________%
2. Amount of lactic acid produced by *L. brevis* is ___________%

Expt. No.02 Date

Sauerkraut fermentation

Aim: To ferment cabbage to produce Sauerkraut

Theory:

Sauerkraut, a combination of the southern German and Austrian words "kraut," meaning cabbage, and "sauer" meaning sour, literally translates to sour cabbage. **Sauerkraut** is finely cut cabbage that has been fermented by various lactic acid bacteria, including *Leuconostoc*, *Lactobacillus*, and *Pediococcus*.

It has a long shelf life and a distinctive sour flavor, both of which result from the lactic acid that forms when the bacteria ferment the sugars in the cabbage. It is created by fermenting cabbage over a long period of time. There are several health advantages to homemade sauerkraut. It's high in enzymes and vitamin C, both of which are lost during the heating and pasteurization of store-bought sauerkraut. It's also low calorie and easy to digest. Sauerkraut is made by a process called lacto-fermentation. There are beneficial bacteria present on the surface of the cabbage and, in fact, all fruits and vegetables. *Lactobacillus* is one of those bacteria, which are the same bacteria found in curd, yogurt and many other cultured products. When submerged in brine, the bacteria begin to convert sugars in the cabbage into lactic acid; this is a natural preservative that inhibits the growth of harmful bacteria. This fermentation process also transforms it into something incredibly tasty and gives it additional health benefits — fermented sauerkraut contains a lot of the same healthy probiotics as a bowl of yogurt.

Many health benefits have been claimed for sauerkraut.

- It is a source of vitamins C, B, and K. The fermentation process increases the bioavailability of nutrients rendering sauerkraut even more nutritious than the original cabbage. It is also low in calories and high in calcium and magnesium, and it is a very good source of dietary fiber, folate, iron, potassium, copper and manganese.
- If unpasteurized and uncooked, sauerkraut also contains live lactobacilli and beneficial microbes and is rich in enzymes. The fiber and supply of probiotics improve digestion and promote the growth of healthy bowel flora, protecting against many diseases of the digestive tract.

- Sauerkraut has been used in Europe for centuries to treat stomach ulcers, and its effectiveness for soothing the digestive tract has been well established by numerous studies.

Requirements:

1. Fresh cabbage
2. Cabbage shredder
3. Containers
4. Thick plastic sheet
5. Sterile beaker
6. Salt (NaCl food grade)
7. Curd sample or culture of Lactobacilli and Leuconostoc

Procedure:

1. Fresh cabbage is taken and outer leaves are removed. It is washed with water.
2. Heads are cut in half. The central hard core is removed.
3. Cabbage is weighed and shredded.
4. Salt is added to the concentration of 2 % and mixed thoroughly.
5. 2 spoonfuls of curd / culture is added for 100gm of cabbage and mixed well.
6. The mixture is compressed moderately. It is covered with sterile plastic sheet and weight is kept up on it to hold the cabbage under the surface of brine. During fermentation. Finally the container is covered with lid and it is incubated at Room temperature for 4 weeks.

Result: Sauerkraut is produced having sour taste and crispy nature.

Expt No. 03 Date

Preservation of potato/onion by UV radiation

Aim: To study the effect of UV on potato and onion for preservation.

Theory:

UV rays are powerful bactericidal agents. Are non-ionizing radiations of 100 to 300 nm wavelength and are absorbed by proteins and nucleic acids leading to photochemical changes and subsequent cell death. The death of microorganisms results from the production of lethal mutations in nucleic acid preventing transcription and DNA replication. U V rays are bactericidal / virucidal in the wave length between 200-290 nm (254 nm is most effective).

Radiation technique makes the food safer to eat by destroying bacteria.

Requrements:

1. Equal sized Potatoes and Onions (16 each)
2. UV chamber

Procedure:

1. Dry and clean potatoes are taken and numbered from 1 to 15.
2. Dry and clean onions are taken and numbered from 1 to 15.
3. One potato and one onion are kept as control (Not exposed to UV).
4. Potatoes and Onions are kept in UV chamber for UV exposure for as shown in observation table.
5. These exposed potatoes and onions are kept undisturbed and are observed everyday for any change (spoilage).

Observation table

Sr. No.	**Time of exposure to UV in seconds**	**No. of days required for spoilage**	
		Potatoes	**Onions**
1	**15**		
2	**30**		
3	**45**		
4	**60**		
5	**75**		
6	**90**		
7	**105**		
8	**120**		
9	**135**		
10	**150**		
11	**165**		
12	**180**		
13	**195**		
14	**210**		
15	**225**		

Result:

As the exposure time to UV increases, the time required for spoilage increases.

Conclusion:

Exposure to UV increases shelf life of potatoes and onions provided utilization of proper dose.

Expt. 04 Date

Production of fermented milk by *Lactobacillus acidophilus*

Aim: To produce fermented milk (acidophilous mlk) by *Lactobacillus acidophilus.*

Theory:

Acidophilus milk, sometimes called sweet acidophilus milk, has *Lactobacillus acidophilus* bacteria added to it, giving it a tangy flavor and thickened texture. This cultured product is usually low in fat and has a longer shelf life than ordinary milk. Many people believe it can benefit digestion and prevent allergies due to the activity of the acidophilus bacteria in the intestines.

The culture process used to make acidophilus milk starts with inoculating sterile milk with the bacteria. Next, the milk is allowed to sit at a warm temperature while the bacteria reproduce. *L. acidophilus* thrives in the mildly acidic environment of milk, consuming some of the lactose in the milk in the process. The tangy flavor of the milk will increase the longer it stays warm. This process is similar to the one used to make yogurt but results in a thinner, drinkable product.

Acidophilus bacteria are considered a probiotic, meaning that they are usually beneficial to human health. Some people believe that consuming products that contain live active cultures can help to treat digestive problems and yeast infections. Acidophilus bacteria is said to increase the number of beneficial intestinal and vaginal bacteria when eaten.

This beverage is used in place of regular cow's milk for some individuals who are lactose intolerant. During fermentation, the bacteria feed on the lactose sugar in the milk, breaking some of it down. For people who are lactose intolerant, that mean that their bodies may have an easier time digesting this milk. Acidophilus milk is only lightly fermented. Some studies also suggest that this type of milk may help lower cholesterol levels.

Requirements:

1. Milk
2. Pure culture of *Lactobacillus acidophilus*
3. 0.1 N NaOH
4. Phenolpthalein indicator

Procedure:

1. The milk is boiled and cooled.
2. It is inoculated with Lactobacillus *acidophilus* culture (1 %).
3. Then it is incubated at 37^0 C for 3 to 4 hours.
4. Lactic acid concentration is checked by titration against NaOH.
5. 5 ml acidophilous milk is taken and 5 ml D/W is added in it.
6. Boiled to drive off CO_2. Then cooled and 2 to 3 drops of phenolphthalein indicator are added.
7. It is titrated against 0.1 N NaOH. (End point colourless to pink).
8. % of lactic acid is calculated by formula

$$\text{\% of Lactic Acid} = \frac{\text{Burette reading X Normality of NaOH X Mol. Wt of Lactic Acid}}{\text{Amount of sample taken X 10}}$$

Mol. Wt of Lactic Acid = 90

Result:

1. % of lactic acid is ____________________________

2. pH of the acidophilous milk is __________________

Conclusion:

Acidophilous milk is successfully produced.