

Production of Lactic Acid

1. Prepare Culture Medium

- 1.1 Solute the following components in 1 L distilled water:

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|------------------------|--------|
| Proteose Peptone No. 3 | 10.0 g |
| Beef Extract | 10.0 g |
| Yeast Extract | 5.0 g |
| D-Glucose | 20.0 g |
| Polysorbate 80 | 1.0 g |
| Ammonium Citrate | 2.0 g |
| Sodium Acetate | 5.0 g |
| Magnesium Sulfate | 0.1 g |
| Manganese Sulfate | 0.05 g |
| Dipotassium Phosphate | 2.0 g |

pH = 6.3 ± 0.2 at 25°C

- 1.2 Autoclave the culture medium at 121 °C for 20 minutes.



2. Prepare Over Night Culture

- 2.1 Inoculate 200 ml of the culture medium with *Lactobacillus delbrueckii*.
- 2.2 Incubate culture at 37 °C over night until there is a visible turbidity.



3. Prepare Fermentation

- 3.1 Inoculate fermenter medium with overnight culture (volume ratio 10 : 1) and measure at t_0 optical density at 600 nm, lactic acid- and glucose concentration at 340 nm.
- 3.2 The fermentation is carried out at:
- 37 °C e.g. in a water bath
 - stirring at 100 rpm
 - pH-controlled in the range of 5.0 – 6.0 for neutralization of produced lactic acid add 2 m NaOH
 - control glucose concentration during fermentation;
- if glucose is consumed, feed medium with a glucose solution of 1 m/L.



4. Fermentation

- 4.1 Ferment for 3 days at 37 °C and a pH of 5.5.
- 4.2 Take frequent samples to measure glucose-, lactic acid concentration and optical density.

