Preparation of Yamanaka Media

Introduction

This protocol creates preferred liquid growth medium for growing *Komagataeibacter rhaeticus* iGEM. Following this protocol will yield 500 mL of Yamanaka + glucose media.

Reagents

- 25 g glucose (10% w/v)
- 2.5 g yeast extract
- 2.5 g ammonium sulfate [$(NH_4)_2SO_4$]
- 1.5 g monopotassium phosphate [KH_2PO_4]
- 0.025 g Magnesium sulfate
- 500 mL Milli-Q H_20
- HCl and NaOH to adjust pH

Equipment

- 2x 500 mL Erlenmeyer flasks
- Analytical balance
- pH probe
- Aluminum foil
- Autoclave

Procedure

1. In one flask, add 25 g of glucose and enough Milli-Q H_2O to fill up to the 250mL mark. Stir vigorously to dissolve.
2. In another flask, add the rest of the ingredients and enough Milli-Q H_2O up to the 250mL mark.
3. Prepare the pH meter by calibrating using pH buffer solutions.
4. Adjust the pH of both flasks using HCl or NaOH to a pH of 5.0.
5. Autoclave both bottles at 121°C for 20 minutes.
    a. The flasks are separate because yeast extract cannot be autoclaved with the glucose; otherwise, the Maillard reaction occurs, introducing toxic byproducts into the media.
6. Remove the flasks from the autoclave and let cool. Pour the contents of one bottle into the other.