Supernatant extraction and purification

Introduction

By culture P. aeruginosa in ABT. AHL will be in the supernatant, so we purify the supernatant and can be using in other experiments.

Reference:
1. Ravn, L., Christensen, A. B., Molin, S., Givskov, M. C., & Gram, L. (2001). Methods for detecting acylated homoserine lactones produced by Gram-negative bacteria and their application in studies of AHL- production kinetics. *Journal of Microbiological Methods*, *44*(3), 239-251.

Materials

- Bacterial strain
- ABT broth
- 0.22um filter

Procedure

ABT preparation

1. Prepare MQ 100ml
2. Add $(NH_4)_2SO_4$ 0.04 g, Na_2HPO_4 0.06 g, KH_2PO_4 0.03g, NaCl 0.03g
3. Add $MgCl_2$ 0.1mmol, $CaCl_2$ 0.01 mmol, $FeCl_3$ 0.001 mmol
4. High-temperature sterilization
5. Add Thiamine 0.25 mg, Glucose 50mg, Casamino acid 50mg

Culture

6. Culture PA in 2ml ABT overnight

Purification

7. Use 0.22um filter to purify the supernatant
8. Spin down 14000 5min