Butyrate Extraction:

Method 1: Solvent Extraction

Method to extract butyrate from cell culture media. Principle of 'back extraction', change charge of SCFAs to make them soluble in aqueous or organic phases and use that to remove impurities. Use this method to prepare SCFA samples for the HPLC. Does not need to be sterile.

- Grow bacteria anaerobically for 48 hours, to trigger butyrate production.

1. Centrifuge culture media:
    a. 2300g for 5 minutes, at 4^{o}C (not essential).
    b. Collect supernatant in glass tube.
2. Add 35µl of concentrated phosphoric acid (in acids box- labelled as orthophosphoric acid):
    a. Vortex for 15 seconds.
3. Add 5ml of diethyl ether (in solvents cupboard):
    a. Use a glass pipette.
    b. Vortex for 15 seconds.
4. Gently roll for 20 minutes:
    a. Use spinning carousel, check it is correct angle and tube won't fall out.
    b. Can parafilm lid to hold it in if needed.
5. Centrifuge (does not needed to be centrifuged, can be left to separate layers):
    a. Transfer top clear layer to another pyrex tube.
    b. Use glass Pasteur pipette to transfer layers (silver drawers).
6. Add 500µl of 1M NaOH:
    a. Dilute down from 10M stock on worktop.
    b. Vortex for 15 seconds.
    c. Gently roll to extract for 20 minutes (same as above).
7. Transfer lower layer phase to the autosampler vial.
    a. Add 35µl of concentrated phosphoric acid (acids box).