Replacement of carbon sources and determination of butyric acid

1. **Culture of *Clostridium tyrobutyricum* engineered bacteria *Ct (Pthl F/Xpk-BD)* using TGY medium supplemented with different carbon sources**
2. **Fermentation broths of Ct (Pthl F/Xpk-BD) in media containing different carbon sources were collected and assayed for product content using HPLC**
3. **Analysis of HPLC data**

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Recorder: Yijiu Lu

7/8/2023 Monday

Steps-*Clostridium tyrobutyricum activation:*

Medium preparation and sterilization: on the basis of TGY medium, glucose was replaced with equimolar amounts of fructose and xylose, respectively, 50mL system

***Clostridium tyrobutyricum* activation:** 1 mL *Ct (Pthl F/Xpk-BD)*, no resistance added, add to pre-prepared TGY medium and incubated for 12 hours.

8/8/2023 Tuesday

Steps-*Clostridium tyrobutyricum secondary activation:*

***Ct(Pthl F/Xpk-BD)*, add double antibody (Tm and Dcyc),50mL, 12 hours**

Inoculation: the activated bacteria were inoculated into the medium with different carbon sources; the initial medium was taken and samples were retained

9/8/2023 Wednesday

Steps-*take the samples:*

Samples of the broth were taken once every 6-8 hours and OD_{600} was measured at the same time.

10/8/2023 Thursday

Steps-*Product Concentration Detection*:

Liquid mobile phase: 267μL of concentrated sulfuric acid in 1L of water, filtration, ultrasonication.

Sample processing: centrifugation, membrane transfer, sample loaded to HPLC

11/8/2023 Friday

Steps-*Record HPLC results:*

Recording of results from HPLC

14/8/2023 Monday

Steps-*Solution preparation:*

1. hydroxylamine solution: 4M hydroxylamine hydrochloride: 3.5M NaOH=1:1,v/v
2. ferric chloride solution: 5% ferric chloride: 12% trichloroacetic acid: 3M HCl=1:1:1,v/v/v

Clostridium tyrobutyricum **activation:** Control; *Ct(Pthl F/Xpk-BD)*; *Ct(Pthl F/Xpk-QS)*, no resistance, 1mL, 12 hours

15/8/2023 Tuesday

Steps-*Clostridium tyrobutyricum secondary activation:*

Control; *Ct(Pthl F/Xpk-BD)*; *Ct(Pthl F/Xpk-QS)*, add double antibody (Tm and Dcyc), 50mL, 12 hours

Medium preparation and sterilization:

on the basis of TGY medium, replace glucose with equal molar amounts of fructose and xylose respectively, 50mL system

Inoculation: the activated bacteria were inserted into the medium.

16/8/2023 Wednesday

Steps-*Measuring acetyl phosphate:*

1. Take the bacteria in the pre-stabilization period, wash them twice with PBS (10mM, pH 7.2-7.4), and ultrasonically break them;
2. Centrifuge the broken cells (12000rpm,5min), and set the supernatant on ice;
3. Take 500 μL of supernatant, add 250 μL of hydroxylamine solution for reaction, and leave it at room temperature for 30 min;
4. Add 750μL ferric chloride solution to develop the color, and leave it at room temperature for 10min, avoiding light;
5. Determine the absorbance value at 540nm.

The data are as follows