Data

Thursday, January 25, 2024 5:38 AM

Input Variables:

1. **Vessel Type**: The type of fermentation vessel used, which could affect the fermentation process due to design features like stirring mechanisms or oxygen transfer capabilities.
2. **Vessel Volume**: The total volume capacity of the fermentation vessel.
3. **Vessel Name**: Identifier for the specific fermentation vessel used.
4. **Production Day**: The day of the production run in the fermentation process.
5. **Timepoint (hr)**: Specific hour(s) during the fermentation process when measurements are taken or actions are performed.
6. **Agitation**: The stirring speed in the fermentation vessel, which affects oxygen transfer and mixing.
7. **DO (Dissolved Oxygen Percentage)**: The amount of oxygen dissolved in the fermentation broth, crucial for aerobic growth of E. coli.
8. **pH Setpoint**: where the pH is set for the experiment.
9. **Gas Flow**: Rate of gas (like air or oxygen) flow into the vessel, important for maintaining oxygen levels and removing carbon dioxide.
10. **Air (%)**: Percentage of air in the gas mixture supplied to the vessel.
11. **O2 (Oxygen percentage):** Percentage of oxygen in the gas mixture.
12. **Temp (Temperature)**: The temperature of the fermentation process, critical for enzymatic activities and growth rate.
13. **Media Type**: The composition of the growth media, which provides nutrients for E. coli growth **(PhoA,** and/or **T7)**.
14. **Feed Type**: The type of feed (like glucose) added to the media during the fermentation.
15. **Glucose Limit**: the lowest concentration of glucose at which to add more glucose.

Output Variables:

1. **Timepoint (hr**), **OD600 (Optical Density at 600 nm)**: Measure of cell density or turbidity of the culture at specific time points.
2. **WCW (Wet Cell Weight) (g/L)**: The weight of cells per liter of culture, indicating cell growth.
3. **Agitation, Air %, DO %, GasFlow, O2, Temp**: Similar to input variables, but these are the actual measured values during the process.
4. **Ph (Potential of hydrogen)**: The actual pH level of the culture. Used to specify the acidity or basicity (alkalinity) of the culture.
5. **Feed %**: Percentage of feed in the culture at specific time points. The feed for this experiment contains 50% glucose solution
6. **Titre (mg/ml)**: Concentration of the desired recombinant protein in the culture.
7. **Glycerol (g/L)**: Concentration of glycerol, which can be used as a carbon source.
8. **Glucose (g/L**): Concentration of glucose in the culture. Glucose is the primary carbon source for the recombinant protein-producing microorganisms like E. Coli. The goal is to keep the glucose less than 20g/L.
9. **Acetate (mmol/L)**: Acetate concentration, an important indicator as high acetate levels can inhibit cell growth, reduce carbon activity, and negatively impact protein folding and stability . Acetate is an unwanted byproduct. When Acetate production is above 5mmol/L, we add more feed.
10. **Phosphate (mmol/L)**: Concentration of phosphate, which is a crucial nutrient.

In a fermentation process like this, controlling and monitoring these variables is critical to optimizing the conditions for maximum yield and quality of the recombinant protein. The input variables are what you can control or set, while the output variables are what you measure or observe as a result of the process conditions.

Combine the following as outcome variables:

NB: data was determined based on the SDS gel page

- OD600– Optical density600
- WCW (g/L) – Wet cell weight - use this in place of titer (mg/ml)
- DO% - Dissolved oxygen percentage
- pH – Potential of hydrogen
- O2 – Oxygen percentage

Each of the excel file is a different experiment conducted on different days, the numbers labelled symbolizes the year/month/date performed. They are all phoA process. So PhoA process I have labelled as Media A. As for now the experiments shared with you will all be Media A.

Control variables (notes taken from lab visit):

1. Feed type
2. pH
3. Temperature
4. DO
5. Induction OD/Feed OD.

Media Type variable (PhoA, T7)

PhoA (Alkaline Phosphatase):

- **Function**: Alkaline phosphatase (PhoA) is an enzyme produced by E. coli and other bacteria. In E. coli, it is encoded by the phoA gene. This enzyme is commonly used in molecular biology and biochemistry.
- **Role in Recombinant Protein Production**: In the context of recombinant protein production, PhoA can be used as a reporter gene or as a tag. When fused to a protein of interest, the PhoA tag can aid in the protein's purification and quantification. Its activity can be easily measured, making it a useful tool for assessing the expression levels of the target protein.
- **Induction**: The expression of PhoA in E. coli is typically regulated by the phosphate concentration in the medium. Under conditions of phosphate limitation, the PhoA enzyme is upregulated.

Reason for missing data:

- We usually don't measure the WCW (g/L) till initiation of feed unless and until it is required. It is a standard practice during runs.

Questions to ask Nikki regarding experiment variables:

1. Feed Changes:

a. Composition of Feed: Other than glucose, are there any other components included in the feed at each time point? If so, what are they and what proportions are used?

Ans: No, only 50% glucose solution.

b. Decision Factors for Feed Changes: Could you describe the criteria or factors that inform how the feed percentage is adjusted throughout the experiment? For instance, what prompts a change from 10.6% to 20.21%, and then to 7.9% at the specified hours?

Ans: Acetate production above 5mmol/L and would like to keep the glucose less than 5g/L.

c. Feed Calculation Variables:

i. How exactly is the 'feed' variable quantified? Is it measured in volume, weight, or concentration?

Ans: Feed rate ml/min, Feed rate ml/min; measured in concentration. Feed is measured using a Cedex to provide concentration of glucose(g/L). The feed rate(mL/min) is set as a in percentage (%) for our pumps. It's calculated from a ratio, as explained in c ii.. Using #14 tubing the rate is 3.4mL/min at 100%. If you want the feed at say 1mL/min...3.4/100=1/x; x=29.4%, The pump would be set at 29.4%.

ii. How is the 'feed %' calculated? Is it relative to the batch volume, or another metric?

Ans: 3.4 ml/min at 100%, do ratio.

iii. The input data shows that 'Glucose' is the only feed type. However, the output section has both columns for 'glucose (g/L)' and 'feed', which could mean that glucose is one of the many components of the feed. Could you share other components of the feed and their proportions?

Ans: No other components.