**140618 QA1-QD3**

**Arrowhead Media DOE**: Biotin, (NH4)2SO4 and FeSO4\*7H20

|  |  |  |  |
| --- | --- | --- | --- |
| **Tank** | **Media** | **Biotin** | **FeSO4\*7H2O** |
| **Q\_A1** | **1.0x KA1 w/ 8.0 g/L (NH4)2SO4** | High | Medium |
| **Q\_A2** | Low | Medium |
| **Q\_A3** | Medium | Low |
| **Q\_B1** | Medium | High |
| **Q\_B2** | **1.0x KA1 w/ 9.5 g/L (NH4)2SO4** | Low | Low |
| **Q\_B3** | High | Low |
| **Q\_C1** | Low | High |
| **Q\_C2** | High | High |
| **Q\_C3** | **1.0x KA1 w/ 11.0 g/L (NH4)2SO4** | Medium | Medium |
| **Q\_D1** | Low | Medium |
| **Q\_D2** | High | Medium |
| **Q\_D3** | Medium | High |

**SEEDs:**

Seed I – 1 x 80mL SP92 (75g/L glu) will be inoculated with 1mL of **sAA3147** glycerol stockand grown in a 500mL baffled flask with a foam plug at 30°C for 24 ± 4 hours.

Seed II – 3 x 80 mL SP92 (75g/L glu) for each strain will be inoculated to a starting OD of 0.4 in a 500mL baffled flask with a foam plug and incubated for 24 ± 4 hours.

**Inoculation:**

1. Sterilize each Q+ reactor with 15mLs DI water.

3. In the hood, **aseptically add 270mLs of the appropriate 1.11x KA1 media** to each reactor then ***add the correct amount of each additional media component*.** Once this is done hook the tanks up to the DCU and turn on agitation, temperature control and airflow to set points.

4. Ensure that the pH process value is ~5.8 and turn on pH control at set point

5. Calculate required seed for an initial OD of 1, add 0.5mLs of MCA460, and add 5% inoculums and make up remaining 15mLs with sterile water.

**Fermentation Conditions:**

Media: **See Table Above**

Initial Volume: 0.30L

Temperature: 35°C

pH: 5.8 (6N NaOH)

Agitation: 1200 RPM

Aeration: 0.30 LPM

Antifoam: 1mL of MCA460 as needed

**Induction:** (DO Spike ~12-14hrs TFT)

Bolus: **None**

Temperature: 30°CAgitation: Max (Set at 1300 RPM)

Aeration: 0.3 LPM

pH: 4.5 @ I0

Primary Feed: **Et-PFAD Batch #006**

Primary Feed Rate: 0.63 g/L-hr I0-I12; 1.35g/L-hr I12-I96

**Sampling:** (See details)

Pull samples directly from the head-plate with a wide-bore serological pipette

LC: 200 µl of unfiltered fermentation broth on 1800µL LCMP; then 0.2µm-filter.

GC: 1mL whole broth on 800µL 6N HCl in a 50mL falcon tube **by weight**

DCW: 1mL on 0.2um cellulose acetate filters

**Termination:**

After final sample is pulled, increase temperature to 60°C. After temperature has reached 60°C, increase pH to 7.0 and swirl tank, then pull post-treat sample.

**Sampling Details:**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Date & Time** | **OD** | **Plate** | **LC** | **GC** | **Scope** | **DCW** | **Rate Check** | **Total** | **Sign off** |
| **Inoculation** | Wed. PM | 0.1 | -- | 0.2 | -- |  | -- | -- | 1.0 |  |
| **I0-** | Thur. AM | 0.1 | -- | 0.2 | 1.0 | 0.05 | 1.1 | -- | 3.0 |  |
| **I12** | Thur. PM | -- | -- | 0.2 | 1.0 | -- | -- |  | 2.0 |  |
| **I24** | Fri. AM | 0.1 | -- | 0.2 | 1.0 | -- | -- |  | 2.0 |  |
| **I48** | Sat. AM | 0.1 | -- | 0.2 | 1.0 | -- | -- |  | 2.0 |  |
| **I72** | Sun. AM | 0.1 | -- | 0.2 | 1.0 | 0.05 | -- |  | 2.0 |  |
| **I84** | Sun. PM | -- | -- | 0.2 | 1.0 | -- | -- | -- | 2.0 |  |
| **I96** | Mon. AM | 0.1 | -- | 0.2 | 1.0 | -- | -- |  | 2.0 |  |
| **I108** | Mon. PM | -- | -- | 0.2 | 1.0 | -- | -- | -- | 2.0 |  |
| **I120** | Tues. AM | 0.1 | 0.1 (-4,-5,-6) | 3x | 3.0 | 0.05 | -- |  | 3.0 |  |
| **Post-Treat** | Tue. AM | -- | -- | 3x | 3.0 | -- | -- | -- | 2.0 |  |

**Schedule:**

|  |  |
| --- | --- |
| Inoculation of Seed I | Mon. 06/16/14 @ ~ 1600 |
| Inoculation of Seed II | Tues. 06/17/14 @ ~ 1700 |
| Inoculation of QA1-D3 | Wed. 06/18/14 @ ~ 2100 |
| Induction of QA1-D3 | Thur. 06/19/14 @ ~ 1000 |
| **I₁₂ Feed Rate Change** | Thur. 06/19/14 @ ~ 2200 |
| Primary Feed Off | Mon. 06/23/14 @ ~ 0900 |
| Termination | Tue. 06/24/14 @ ~ 0900 |

**Seed II & Inoculation Volumes:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Strain** | **Seed II ODF** | **Seed Vol Req’d**  **(mL)** | **Water Vol Req’d**  **(mL)** |
| **sAA3147** |  |  |  |

**Background:**

We will be repeating this run to gather duplicate data for this experiment…

Continuing the Media DOE for Arrowhead, we will be narrowing in on 3 media components: Biotin, Ammonium Sulfate [(NH4)2SO4] and Ferrous Sulfate (FeSO4\*7H20). There will be 3 stock solutions of 1.0x KA1 with different concentrations of (NH4)2SO4 then high, medium or low concentrations of Biotin and FeSO4\*7H2O will be added to each tank per the table below:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Run** | **Tank** | **Pattern** | **Biotin (μL)** | **FeSO4\*7H2O (μL)** | **(NH4)2SO4 (g/L)** | **Water (μL)** |
| **1** | **A1** | +0- | 450 | 600 | 8 | 300 |
| **2** | **A2** | −0− | 150 | 600 | 8 | 600 |
| **3** | **A3** | 0−− | 300 | 300 | 8 | 750 |
| **4** | **B1** | 0+− | 300 | 900 | 8 | 150 |
| **5** | **B2** | −−0 | 150 | 300 | 9.5 | 900 |
| **6** | **B3** | +−0 | 450 | 300 | 9.5 | 600 |
| **7** | **C1** | −+0 | 150 | 900 | 9.5 | 300 |
| **8** | **C2** | ++0 | 450 | 900 | 9.5 | 0 |
| **9** | **C3** | 0−+ | 300 | 300 | 11 | 750 |
| **10** | **D1** | −0+ | 150 | 600 | 11 | 600 |
| **11** | **D2** | +0+ | 450 | 600 | 11 | 300 |
| **12** | **D3** | 0++ | 300 | 900 | 11 | 150 |