**140506 300L**

Test higher feed rate, temperature, ammonium hydroxide instead of sodium hydroxide and 50% reduced ammonium sulfate

|  |  |
| --- | --- |
| **sAA2178** | Δpox4, Δpox5, CPRB+4, P450A19+8 , ADH2a+8, ADH8+7, HFD2+5 |

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Primary Feed**  **Rate I0-I24** | **Primary Feed**  **Rate I24-I132** | **Secondary Feed**  **Rate I0-I133** |
| **300L** | 1.125g/L-hr | **2.30g/L-hr** | **1.62g/L-hr Dextrose** |
| 0.225kg/hr | **0.461kg/hr** | **0.648kg/hr 50% Dextrose (w/w)** |
| 1.340L/hr | **???L/hr** | **0.184L/hr (accounts for backpressure)** |

**SEEDs:**

Seed I –1 x80 mLs SP92 (75g/L Dextrose) will be inoculated with a glycerol stock of sAA2178 in a 500mL baffled flask with a foam plug and incubated for 24 ± 4 hrs at 30C and 250RPM.

Seed II – 80mLs SP92 (75g/L Dextrose) in 3 x 500mL baffled flask with a foam plug will be inoculated to a starting OD of 0.4 and incubated for 24 ± 4 hrs at 30C and 250RPM.

Seed III – 1.5L SP92 (75g/L Dextrose) in 3 x 2L high yield baffled flasks will be inoculated to a starting OD of ~2 covered with a filter and incubated for 24 ± 4 hrs at 30C and 250RPM.

**Fermentation Conditions:**

Media: **1.5x KA1** (4.05% Dextrose, **5.25g/L ammonium sulfate**)

Initial Volume: 200L

Temperature: **34C**

Pressure: 1barg

pH: 5.8, **NH4OH**

Agitation: 500 RPM

Aeration: 200L/min Use Mass Flow Controller

Antifoam: 50mL of AF204 and as needed

Please verify that the pH of the media post-sterilization is around 4.0-4.5 both online and offline. If the pH is out of this range, do not proceed, inform senior operators

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

Bolus: 100mL of antifoam at induction

Temperature: **34C**

Pressure: 1barg

Aeration: 200L/min Use Mass Flow Controller

pH: 6.0

Primary Feed: Ethyl Laurate

Primary Feed Rate: 1.125g/L-hr I0-I24; **2.30g/L-hr I24-I132****Please add 100mL of antifoam at rate change**

Secondary Feed: **50% Dextrose (w/w)**

Secondary Feed Rate: **1.62g/L-hr I0-I133**

**Sampling:** (See details)

Pre-Induction: Measure every 3 hrs until dextrose is < 10g/L by YSI, then every 1hr for YSI only. Continue to take full samples every 3 hours until induction during this phase.

LC: 0.2 ml fermentation broth in 1.8mL LCMP (excluding the initial sample, **dilute first, then filter**), vortex for > 15sec then back fill a syringe and filter into a LC vial.

GC: Tare a 50mL Falcon tube with 0.8mL 6N HCl, add 1mL whole broth directly from the head plate and weigh. Record both the tare weight and the total weight on the logsheet.

DCW: 1mL on pre-dried and pre-weighed 0.2um cellulose acetate filters

Glycerol Stock: 0.6mL whole broth on 0.4mL 50% Glycerol in a cryo vial

IC: 50mL x 2, spun down at max RPM for 5 minutes, decant, store supernatant in cold room

Viscosity: Please follow written protocol

**Termination:**

Prior to final sample, pause primary and secondary feeds

**For final GC samples. Please pull 3 separate samples from the 300L (i.e. 3, 50mL falcons) and perform 1GC assay for each sample rather than 1 sample from the 300L and then 3 samples from that.**

Prior to termination, verify that the glucose by YSI is reading 0. Do not increase temperature until you get a YSI glucose reading of < 0.02g/L

If the YSI reads < 0.02g/L, increase temperature to 60C. After 1 hour at 60C, please harvest the tank into steel drums and store in the cold room.

**Sampling Details:**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Date & Time** | **OD** | **Viscosity** | **Plate** | **IC** | **LC** | **GC** | **Scope** | **DCW** | **Glycerol**  **Stock** | **Rate Check** | **Total** | **Sign off** |
| **Inoculation** | Tues. PM | 0.2 | -- | -- | 100 | 0.1 | -- | -- | -- | -- | -- | 101.0 |  |
| **+3hrs TFT** |  | 0.2 | -- | -- | -- | 0.1 | -- | -- | -- | -- | -- | 1.0 |  |
| **+6hrs TFT** |  | 0.2 | -- | -- | -- | 0.1 | -- | -- | -- | -- | -- | 1.0 |  |
| **+9hrs TFT** |  | 0.2 | -- | -- | -- | 0.1 | -- | -- | -- | -- | -- | 1.0 |  |
| **+12hrs TFT** |  | 0.2 | -- | -- | -- | 0.1 | -- | -- | -- | -- | -- | 1.0 |  |
| **+15hrs TFT** |  | 0.2 | -- | -- | -- | 0.1 | -- | -- | -- | -- | -- | 1.0 |  |
| **I0-** | Wed. AM | 0.2 | -- | 0.2 (5,-6,-7) | 100 | 0.1 | 1.0 | 0.05 | 1.1 | -- | -- | 103.0 |  |
| **I6** | Wed. PM | 0.2 | -- | -- | -- | 0.1 | 1.0 | -- | -- | -- | **YES** | 2.0 |  |
| **I12** | Wed. PM | 0.2 | -- | -- | -- | 0.1 | 1.0 | -- | -- | -- | **YES** | 2.0 |  |
| **I18** | Thur. AM | 0.2 | -- | -- | -- | 0.1 | 1.0 | -- | -- | -- | **YES** | 1.0 |  |
| **I24** | Thur. AM | 0.2 | -- | 0.2 (5,-6,-7) | -- | 0.1 | 1.0 | 0.05 | -- | -- | **YES** | 2.0 |  |
| **I30** | Thur. PM | 0.2 | -- | -- | -- | 0.1 | 1.0 | -- | -- | -- | **YES** | 2.0 |  |
| **I36** | Thur. PM | 0.2 | -- | -- | -- | 0.1 | 1.0 | -- | -- | -- | **YES** | 2.0 |  |
| **I42** | Fri. AM | 0.2 | -- | -- | -- | 0.1 | 1.0 | -- | -- | -- | **YES** | 2.0 |  |
| **I48** | Fri. AM | 0.2 | 500 | 0.2 (5,-6,-7) | -- | 0.1 | 1.0 | 0.05 | -- | -- | **YES** | 502.0 |  |
| **I54** | Fri. PM | 0.2 | -- | -- | -- | 0.1 | 1.0 | -- | -- | -- | **YES** | 2.0 |  |
| **I60** | Fri. PM | 0.2 | -- | -- | -- | 0.1 | 1.0 | -- | -- | -- | **YES** | 2.0 |  |
| **I66** | Sat. AM | 0.2 | -- | -- | -- | 0.1 | 1.0 | -- | -- | -- | **YES** | 2.0 |  |
| **I72** | Sat. AM | 0.2 | 500 | 0.2 (5,-6,-7) | 100 | 0.1 | 1.0 | 0.05 | -- | -- | **YES** | 602.0 |  |
| **I78** | Sat. PM | 0.2 | -- | -- | -- | 0.1 | 1.0 | -- | -- | -- | **YES** | 2.0 |  |
| **I84** | Sat. PM | 0.2 | -- | -- | -- | 0.1 | 1.0 | -- | -- | -- | **YES** | 2.0 |  |
| **I90** | Sun. AM | 0.2 | -- | -- | -- | 0.1 | 1.0 | -- | -- | -- | **YES** | 2.0 |  |
| **I96** | Sun. AM | 0.2 | 500 | 0.2 (5,-6,-7) | -- | 0.1 | 1.0 | 0.05 | -- | -- | **YES** | 502.0 |  |
| **I102** | Sun. PM | 0.2 | -- | -- | -- | 0.1 | 1.0 | -- | -- | -- | **YES** | 2.0 |  |
| **I108** | Sun. PM | 0.2 | -- | -- | -- | 0.1 | 1.0 | -- | -- | -- | **YES** | 2.0 |  |
| **I114** | Mon. AM | 0.2 | -- | -- | -- | 0.1 | 1.0 | -- | -- | -- | **YES** | 2.0 |  |
| **I120** | Mon. AM | 0.2 | 500 | 0.2 (5,-6,-7) | -- | 0.1 | 1.0 | 0.05 | -- | -- | **YES** | 502.0 |  |
| **I126** | Mon. PM | 0.2 | -- | -- | -- | 0.1 | 1.0 | -- | -- | -- | **YES** | 2.0 |  |
| **I132** | Mon. PM | 0.2 | 500 | 0.2 (5,-6,-7) | 100 | 0.1 | **3.0** | 0.05 | -- | 1.0 | **YES** | 605.0 |  |
| **Total** | | | | | | | | | | | | | **~3000mLs** |

**Schedule:**

|  |  |
| --- | --- |
| Inoculation of Seed I | Sun. 5/04/14 @ ~ 0700 |
| Inoculation of Seed II | Mon. 5/05/14 @ ~ 1900 |
| Inoculation of Seed III | Tues. 5/06/14 @ ~ 0700 |
| Inoculation of 300L | Tues. 5/06/14 @ ~ 2100 |
| Induction of 300L | Wed. 5/07/14 @ ~ 1000 |
| **I24 Feed Rate Change** | **Thur. 5/08/14 @ ~ 1000** |
| **Turn off Primary Feed** | **Mon. 5/12/14 @ ~ ????** |
| **Turn off Secondary Feed** | **Mon. 5/12/14 @ ~ ????** |
| Termination | Tues. 5/13/14 @ ~ ???? |

**Seed I & Seed II Inoculation Volumes:**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Strain** | **Seed I ODF** | **Seed I Vol Req’d (mL)** | **Seed II ODF** | **Seed II Vol Req’d**  **(mL)** | **Seed III ODF** |
| **sAA2178** |  |  |  |  |  |

**Background:**

There will be a different “handheld” DO meter hooked up to the 300L. Please read the DO2% from this (it is blue) rather than from the DCU. This one should not have issues with the power supply or running out of room for data collection.

In an effort drive the titer up higher than 140g/L, we may continue this fermentation beyond 132 hours. Please follow up with senior personnel on Monday 5/12 to see when the termination will be scheduled.

With Darwin, feel free to be more liberal with the antifoam rather than controlling exclusively with aeration. In successful Darwin fermentations, antifoam does reduce the foaming more than just temporarily. As always, do whatever is necessary to prevent foamouts.

Testing increased temperature and 20% increased primary and secondary feed rates. These have all worked well small scale so hopefully it is an easy transfer to the 300L.

We will also be testing ammonium hydroxide as a replacement for NaOH. Please be careful, if you smell ammonia when you first walk into the pilot plant, please leave immediately. Find someone who has a respirator to investigate the problem. If you find yourself without a respirator equipped person and are having an issue, please call me and I will come in to help.

**Fermentation Outline:**

|  |  |  |
| --- | --- | --- |
| **Outline Topic** | **Outline Item** | **300** |
| **General** |  |  |
|  | **Rundate** | 140506 |
|  | **Strain** | sAA2178 |
|  | **Brief Unique Identifier** | **pH 4.0 @ I24 #1** |
|  | **Initial Volume (L)** | 0.350 |
|  | **Media** | 2x KA1 |
|  | **Initial Carbon Source** | Glucose |
|  | **Carbon Source Concentration** | 5.40% |
|  | **Inoculum %** | 5 |
|  | **Initial OD Target** | 1 |
|  | **Comments** |  |
|  |  |  |
| **Growth Phase** |  |  |
|  | **Temperature** | 35 |
|  | **Agitation Mode** | Auto |
|  | **Agitation (RPM)** | 1200 |
|  | **Airflow Mode** | Auto |
|  | **Airflow (L/min)** | 0.35 |
|  | **DO Mode** | Off |
|  | **DO Setpoint (%)** | 0 |
|  | **pH Setpoint** | 5.8 |
|  | **pH Deadband** | 0.05 |
|  | **Type of Base** | 6N KOH |
|  | **Growth Feed Mode** | None |
|  | **Growth Feed Substrate** | None |
|  | **Growth Feed Rate** | None |
|  | **Oxygen Supplementation %** | None |
|  |  |  |
| **Induction** |  |  |
|  | **Condition For Induction** | DO Spike |
|  | **Bolus Substrate** | Oleic Acid |
|  | **Size of Bolus** | 10g/L |
|  |  |  |
| **Post-Induction** |  |  |
|  | **Temperature** | 30 |
|  | **Agitation Mode** | Auto |
|  | **Agitation (RPM)** | 1270 |
|  | **Airflow Mode** | Auto |
|  | **Airflow (L/min)** | 0.5 |
|  | **DO Mode** | Off |
|  | **DO Setpoint (%)** | 0 |
|  | **pH Setpoint** | pH 4.0 @ I24 |
|  | **pH strategy** | Constant |
|  | **Primary Feed** | Oleic |
|  | **Primary Feed Rate (g/L/hr)** | 2.25 |
|  | **Primary Feed Strategy** | Constant |
|  | **Primary Feed Start Time** | I0 |
|  | **Primary Feed End Time** | I72 |
|  | **Secondary Feed** | none |
|  | **Secondary Feed Rate (g/L/hr)** | N/A |
|  | **Secondary Feed Strategy** | N/A |
|  | **Secondary Feed Start Time** | N/A |
|  | **Secondary Feed End Time** | N/A |
|  | **Oxygen Supplementation %** | None |
|  |  |  |
| **AA Analysis** |  |  |
|  | **Primary Feed Start Time (hrs)** | 14.5 |
|  | **Primary Feed End time (hrs)** | 32.5 |
|  | **Fermentation End Time (hrs)** | 156 |
|  | **Primary Feed Consumed (g)** | 45946.34 |
|  | **LCAA Final (g/L)** | 0 |
|  | **LCAA Produced (g)** | 0.00 |
|  | **TPAA Produced (g)** | 27974.98 |
|  | **LCAA % Ymax** | 0.000 |
|  | **TPAA % Ymax** | 1.178 |
|  | **LCAA Productivity (g/L-hr)** | 0.000 |
|  | **Specific Productivity (g/L-hr/g/L DCW)** | 0.0000 |
|  | **Purity %** | 0.000 |