STANDARD OPERATING PROCEDURE
FOLEY PILOT PLANT

TITLE: Parallel 2-L Control SScF

AUTHOR: **Ismael U. Nieves** **DATE: March 31st, 2011**
APPROVALS: **Process Change Committee** **DATE:**
HHSM **DATE:**

CONTENTS

A. Scope

This procedure describes the methods for the start of a parallel 2-L SScF to serve as a control for the 80-L SScF.

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B. Safety and Training Requirements

Eye protection and gloves should be worn when dealing with biological medium or basic solutions.

C. Related Documents and SOPs

1. Liquefaction SOP
2. 3-L Bioflo manual
3. Postal balance manual
4. Peristaltic pump manual
5. Water heater manual
6. Bioflo 110 manual

D. Preparation/Materials/Equipment

1. 5 N ammonium hydroxide solution
2. Water bath
3. Water heater
4. Postal balance
5. Peristaltic pump
6. Base titration
7. Bioflo 110 system
8. Sharpie

E. Detailed Procedure

1. Calculate the amount of biomass necessary for every 2-L fermentation (assume 1 L slurry = 1kg slurry):

$$kg\ DW\ =\ 2\ kg\ \text{slurry} * \%\ \text{solids}$$

$$kg\ \text{biomass} = \frac{kg\ DW}{\%DW}$$

2. Approximate the weight of hydrolysate:

$$kg\ \text{Hz}\ = kg\ \text{biomass} - kg\ DW$$

3. Calculate the amount of 5 N ammonium hydroxide that was added to raise the pH to 5.0 for a 2-L fermentation:

$$L\ \text{ammonium hydroxyde} = kg\ Hz * \text{base titration}$$

4. Calculate the amount of biocellulase cocktail that was added for every 2-L SScF (usually 10% of the dry weight):

$$L\ \text{biocellulase} = \%\ \text{to be used}\ * kg\ DW$$

5. Calculate the amount of β-glucosidase that was added for every 2-L SScF:

$$L\ \beta\text{-glucosidase} = L\ \text{biocellulase} * 0.1$$

6. Calculate the amount of water to add for every 2-L SScF:

$$kg \text{ water} = 2\ L - kg \text{ biomass} - L \text{ ammonium hydroxyde} - L \text{ biocellulase} - L\ \beta\text{-glucosidase} - \left(\frac{2\ L - kg\ Hz}{50}\right) - 2 * (2\ L * 0.0015) - (2\ L * 0.05)$$

7. Calculate the amount of slurry to add from the liquefaction step:

$$kg \text{ slurry to add} = kg \text{ water} + L \text{ ammonium hydroxide} + kg \text{ biomass} + L \text{ biocellulase} + L\ \beta\text{-glucosidase}$$

8. Using a postal balance and the peristaltic pump, add the calculated amount of slurry into the 3-L Bioflo.
9. Make sure the Bioflo 110 parameters have been set to the desired conditions (pH and RPM), the pH probe has been calibrated and sterilized, and that the water heater is turned on and up to the desired temperature.
10. Place the 3-L reactor in the water bath and connect the equipment (impeller motor, temperature probe, pH meter) as instructed in the Bioflo 110 manual.
11. After the temperature and pH have been adjusted, add the media components.
12. Inoculate the fermentation. Take a sample right after inoculation in two 1.5-mL microcentrifuge tubes (sample t = 0 h), centrifuge the sample, and freeze the supernatant (-20 °C) for further analysis.

F. Data Archival and Analysis

Record the data in the Parallel 2-L Control Log and store in the Batch Log Book.

G. Tickets

Parallel 2-L Control Log

Date		% Solids	
%DW Biomass		Biomass per 2-L (kg)	
Ammonium Hydroxide Titration to pH 5.0		DW Biomass per 2-L (kg)	
Weight of hydrolysate per 2-L (kg)		Biocellulase Cocktail Used	
Biocellulase Added per 2-L (L)		β-Glucosidase Added per 2-L (L)	
Ammonium Hydroxide Added per 2-L (L)		Water Added per 2-L (kg)	
Slurry Transferred to Bioflo (kg)			