STANDARD OPERATING PROCEDURE
FOLEY PILOT PLANT

TITLE: Liquefaction

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APPROVALS: **Process Change Committee** **DATE:**
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A. Scope

This procedure describes the methods for biomass liquefaction (saccharification) once it has been pretreated.

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B. Safety and Training Requirements

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

C. Related Documents and SOPs

1. Biomass pretreatment SOP
2. OHAUS 5000 Series Xtreme W balance manual
3. Denver Instruments balance operation manual
4. Dry weight by loss on drying SOP
5. Ammonium hydroxide titration
6. 140-L Applikon fermentor manual
7. Postal balance manual
8. Peristaltic pump manual

D. Preparation/Materials/Equipment

1. High pressure boiler
2. 5 N ammonium hydroxide solution
3. Sharpie
4. Denver Instruments balance
5. Biocellulase cocktail
6. Novozyme 188 β-glucosidase
7. Pretreated biomass
8. Autoclaved water in 140-L Applikon fermentor
9. Sterilized tubing
10. 250-mL sample bottle with cap
11. Sterile ladle
12. Postal balance
13. Peristaltic pump
14. Sterile peristaltic pump tubing

E. Detailed Procedure

1. Make sure the Univat tank has been sterilized, rinsed, and is up to temperature.
2. Calculate the amount of biomass to be liquefied in terms of dry weight:

$$kg\ DW = kg\ \text{pretreated biomass} * \%DW$$

3. Calculate the total weight of the slurry if fermenting all the pretreated biomass:

$$\text{total weight} = \frac{kg\ DW}{\%\ \text{solids}}$$

4. Approximate the weight of hydrolysate present in the pretreated biomass:

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

5. Calculate the amount of 5 N ammonium hydroxide to be added to raise pH to 5.0:

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

6. Calculate the amount of biocellulase cocktail to add (usually 10% of the dry weight):
$$L \text{ biocellulase} = \% \text{ to be used } * kg\ DW$$

7. Calculate the amount of β-glucosidase to add:
$$L\ \beta\text{-glucosidase} = L \text{ biocellulase} * 0.1$$

8. Calculate the amount of water to add:
$$kg \text{ water} = \text{total weight} - kg \text{ pretreated biomass} - L \text{ ammonium hydroxyde} - L \text{ biocellulase} - L\ \beta\text{-glucosidase} - \left(\frac{\text{total weight} - kg\ Hz}{50}\right) - 2 * (\text{total weight} * 0.0015) - (\text{total weight} * 0.05)$$

9. Connect the sterile tubing to the Applikon fermentor containing the autoclaved water.
10. Note the weight in the fermentor console.
11. Using the inside pressure of the fermentor, add the water needed to the Univat (minus 2 kg) by difference in weight.
12. Turn on the impeller of the Univat.
13. Add the ammonium hydroxide.
14. Add the pretreated biomass.
15. Allow for the biomass to equilibrate for ~15 min. Take a sample into a 250-mL sample bottle using sterile ladle. This will be the t = -6 h sample. Store the sample in the refrigerator.
16. Dilute the biocellulase and β-glucosidase in the remaining 2 L of water and add the diluted enzyme to the biomass slurry.
17. After 6 h, proceed to transfer the slurry (by weight) to the 140-L fermentor using the peristaltic pump and the corresponding sterilized tubing.
18. Using a postal balance, transfer by weight some of the slurry into a parallel 3-L Bioflo as a 2-L control.
19. Take a sample into another 250-mL sample bottle. This will be sample t = 0 h for the liquefaction.
20. Measure dry weight by loss on drying.

F. Data Archival and Analysis

Record the data in the Liquefaction Log and store in the Batch Log Book.

G. Tickets

Liquefaction Log

Date		% Solids Wanted	
%DW Pretreated Biomass		Pretreated Biomass (kg)	
Ammonium Hydroxide Titration to pH 5.0		DW Pretreated Biomass (kg)	
Weight of hydrolysate (kg)		Biocellulase Cocktail Used	
Biocellulase Added (L)		β-Glucosidase Added (L)	
Ammonium Hydroxide Added (L)		Water Added (kg)	
%DW After Liquefaction			