Campaign 12

Operations Summary

Stan Mayfield Biorefinery
Cellulosic Research and Demonstration Plant

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Operations - Campaign 12
04/06/2015 – 04/12/2015

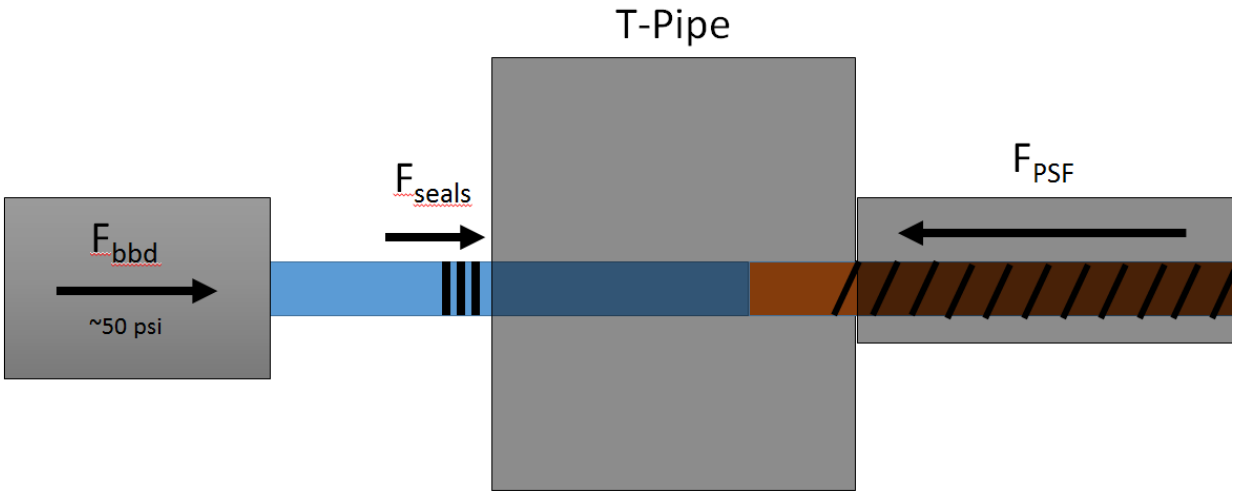
Experimental plan notes:

1. Propagation hydrolysate: 30% 185C
2. Pretreatment temperature: 185C
3. Liquefaction: 15% solids with 5% enzyme.
4. Resorting back to the larger scale fermentation. Using a less toxic hydrolysate given the troubles with the seed train in the past few campaigns, and also using the traditional 3-step seed train (flasks into 2B into 3B). Inoculation into the fermentor will be different though – slurry will be added to the propagator 3B seed when it's ready. Once the level reaches 800 gallons in propagator 3B, all will be transferred into 800 gallons of slurry in the fermentor. This method should present less of a shock to the cells, and will allow them to adapt to the slurry more easily.

Operation problems & resolutions:

1. Pretreatment chute clogging and worn plug screw
    a. <u>Problem:</u>
        i. The chute clogging has been a reoccurring problem for several months, but we believe we finally know the reason. We had to run the speed of the plug screw at a much higher speed than usual during this campaign, indicating that the flights had worn. See past operation summaries for further detail into what the implications of a clogged chute are.
    b. <u>Resolution:</u>
        i. The plug screw is worn out again. See pictures below. We had put about 1000 hours on the screw since we got it resurfaced. The worn flights likely do not move the biomass uniformly which causes the biomass to back up and clog the chute. We had originally thought that if the clogging was from a worn plug screw, it would be evident from the amps, but apparently it was not. We had been hesitant to remove the screw because we were worried of damaging equipment in the process, but the operators are much more comfortable doing so after this diagnosis. We will be watching this screw closely, and we have already set the wheels in motion to have a brand new screw fabricated. We will install the new screw as soon as it arrives because it will allow us to run at a higher biomass flow rate again. In the meantime, we will continue running the worn screw, but we are going to keep the dampner pressure low and limit the hours ran.
        ii. The plug screw wears over time due to the friction force from the plug created by the blow back dampner, but we considered a couple other possible forces which might have contributed to the rapid wear of the plug screw. For one, the seals around the blow back dampner shaft seem to expand when really hot which gives a friction force against the shaft. The blow back dampner shaft is

quick to respond when the system is cool, but it is very slow when the system is hot and the seals have expanded. This friction force from the seals on the shaft might add to the total force the plug screw is experiencing. See the free-body diagram below. The second possible contribution to wear is the effect vibrations from the mechanical vibrator have on the screw. The plug screw surely feels the vibrations because all surrounding metal does, and maybe these vibrations in the plug cause the biomass to act like a sander power tool on the flights.

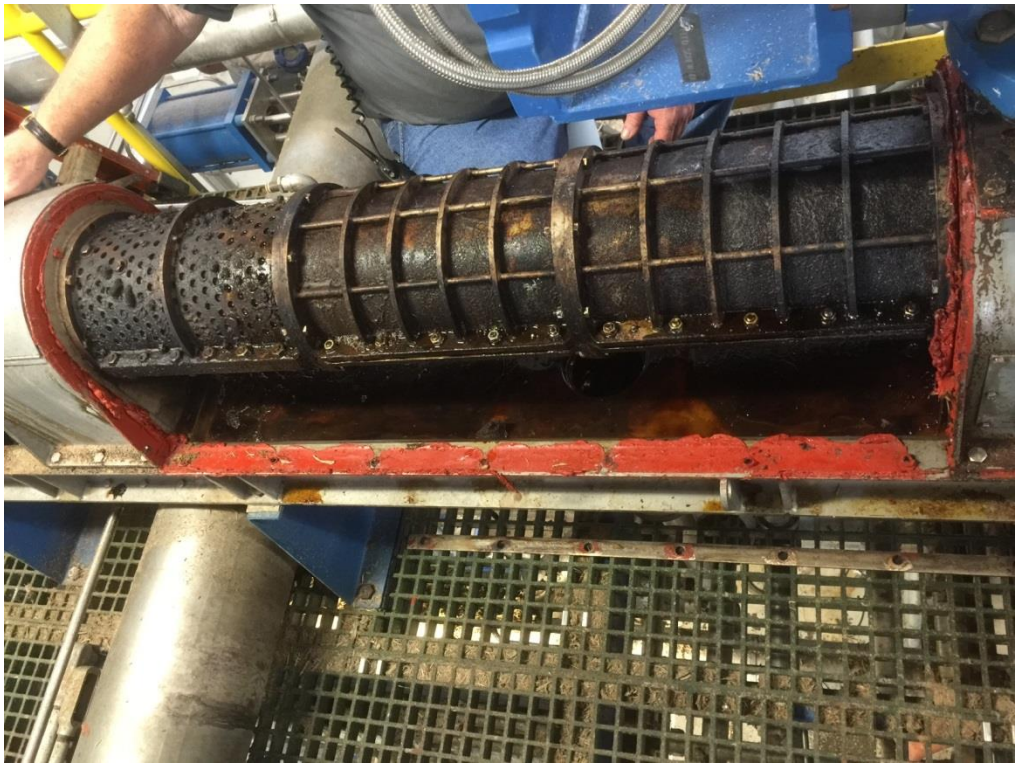


$$\sum F = F_{BBD} + F_{seals} + F_{PSF}$$

$$F_{eq.} = F_{PSF} = F_{BBD} + F_{seals}$$

c. <u>Status:</u>
    i. In progress.

2. Squeezing hydrolysate
    a. Problem:
        i. We disposed of our stock 190C hydrolysate after the failure in campaign 11, so we needed to squeeze a fresh batch while running at the new pretreatment temperature (185C). Unfortunately, squeezing with the metso screw press was incredibly slow, and it seemed like we were overworking the mechanical screw press and screw press feed screw in the process. We initially thought the reason for the slow squeezing was due to fouling/clogging of the screw press screen (see picture below). However, after cleaning the screen with a pressure washer, the squeezing rate was still slow. The likely reason for the slow squeezing was the high dry-weight of the cake, approximately 37-40%, and the fact that the severity of the pretreatment was lower than when at 190C.



b. Resolution:
    i. We set-up a system to use the small portable screw press to squeeze hydrolysate. See picture below. The rate of squeezing was about 7 gallons per hour (back pressure of 40psi), which was much greater than what we were getting from the Metso screw press. The small screw press was also much more efficient than the Metso screw press, which meant far less cake was needed to squeeze a certain amount of hydrolysate. However, the small screw press could not squeeze at a fast enough rate to keep pace with the flow of cake out from the pretreatment system. To limit the amount of hours ran on the pretreatment system and to conserve biomass, we decided to collect excess cake on a clean tarp for squeezing. See picture below. This method of squeezing ended up being ideal and effective.

c. Status:
    i. Resolved, but the small screw press is only a temporary fix. We are considering adding water to the cake before it reaches the screw press to decrease the dry-

weight and allow for a better squeeze, but obviously this comes at a cost since you will be diluting the sugars.

3. Hydrolysate spill
    a. <u>Problem:</u>
        i. The week prior campaign 12, we squeezed approximately 180 gallons of 185C hydrolysate (using the small screw press on the floor) and collected it in a large plastic tote. The hydrolysate was tested in preparation for the campaign, and the results were a success. Unfortunately, on the first day of the campaign, immediately before we started preparing the propagator 2 with hydrolysate, the drain valve on the hydrolysate tote broke and the entire contents drained to floor.
    b. <u>Resolution:</u>
        i. In order to stay on schedule, we had to squeeze another 150 gallons of hydrolysate as quickly as possible. We started pretreatment and started squeezing using the small screw press method described in problem 2. We also rigged up a new drain and secured it to ensure we would not lose it all again. See picture below.
        ii. 
    c. <u>Status:</u>
        i. Resolved.
4. Seed timing
    a. <u>Problem:</u>
        i. This has been a reoccurring problem due to the unreliability of our pretreatment system. In this campaign, the pretreatment system was shut down twice – once in the beginning and once at the end. The shutdown in the beginning was the one which caused the poor seed timing. The oretreatment chute clogged (another reoccurring problem – see problem 1 for more detail), and it caused a delay in starting liquefaction. The experimental plan called to partially fill the fermentor with slurry before adding it to the propagator 3B, but due to the delay we had to add slurry to the propagator 3B before adding any to

the fermentor. Once 3B was to the 800 gallon mark, it had to wait until we pumped 800 gallons into the fermentor before it could be transferred. Unfortunately, filling the fermentor to 800 gallons took too long, and the cells in propagator 3B started to die.

b. Resolution:
    i. We decided that for the next campaign we would start liquefaction very early. In this case, we would have plenty of excess time if a problem with pretreatment caused us to shut down. The retention time for liquefaction would be much longer than 6 hours, but Dr. Ismael Nieves said this was fine. See the experimental plan documents for further detail.
c. Status:
    i. In progress.

5. No xylose consumed
    a. Problem:
        i. All glucose was consumed in the 4000 gallon fermentation, but hardly any xylose was consumed. Dr. Ismael Nieves is pretty sure that the reason was because the cells in propagator 3B started dying and went into survival mode. This will alter their metabolic pathways such that consumption of C5 sugars is hindered.
        ii. Dr. Marco Fernandez is concerned the airflow through the sparger might not be adequate.
    b. Resolution:
        i. Better seed timing (already discussed) should fix it. Tests in the lab can be done to see if the air is making a difference.
    c. Status:
        i. In progress.
6. Enzyme in flask
    a. Problem:
        i. Prior to the campaign, an experiment was performed to see if adding enzymes to the flasks (first stage of the seed train) would help liberate more sugar and improve the seed. The 185C hydrolysate contains a relatively high concentration of oligomers, which the enzyme could monomerize. Unfortunately, the enzyme unknowingly reduced the buffer capacity of the seed solution such that the pH dropped and killed the cells before they could begin to grow.
    b. Resolution:
        i. We believe adjusting the pH after the enzymes are added to the flask will allow for good growth, but we have not yet tested it.
    c. Status:
        i. Not resolved, but acceptable.
7. Propagator 2B sterile sample port leak.
    a. Problem:

i. The sterile sample port diaphragm for the propagator 2B broke while performing SIP on the tank. See picture below.

b. Resolution:
   i. We had recently added steps in all relevant SOPs to open the SSP while performing SIP on process vessels since we wanted to be over-cautious with contamination prevention, but the steps must now be removed.
c. Status:
   i. Not yet removed from all SOPs, but the team understands the change and ensures that it doesn't happen.

8. Debris in biomass
   a. Problem:
      i. The bagasse load delivered to the biorefinery on 03/30/2015 (batch 10029) was full of debris. The debris included pieces of wood, plastic, metal, and fabric. Our feed system clogged up twice before we realized how much debris was in the pile.
   b. Resolution:
      i. We had to sort through all 13 tons to ensure there was no more debris.
   c. Status:
      i. Resolved

9. Nutrient addition to the fermentor
    a. Problem:
        i. This was the first campaign using the new stainless steel nutrient totes which were fabricated with level indicators so we could accurately add nutrients to the fermentors. See picture below.
        ii. The sight level indicators are polycarbonate and are rated for high pressure and temp, and we pressure and leak tested the totes prior to the campaign, but we still experienced leaking while pumping the nutrients into fermentor C. We heated up the totes with steam prior to filling them with nutrients, and we believe that during this process the plastic deformed and then reformed with a weaker seal.
        iii. We also noticed a small leak in the trace metals line underneath the metal walk-way about ¾ of the way into the fermentation. Ismael said it was not enough to make a difference though.

    b. Resolution:
        i. We took the nutrient level indicators out and applied some high temperature silicone at the top and bottom connections.
    c. Status:
        i. We have not yet tested for leaks after a steam heating. The leak under the walk-way has been fixed.

10. Pretreatment blow chamber valve actuator.
    a. Problem:
        i. The bottom ball actuator valve for the pretreatment blow chamber started to malfunction near the end of the campaign. The valve would cycle but the indicator on the actuator which communicates with the HMI would not change position.
    b. Resolution:
        i. The shaft for the actuator indicator was broken. A new one was fabricated and installed.
    c. Status:
        i. Resolved.
11. Clogged C5 pump
    a. Problem:
        i. When preparing hydrolysate for propagator 3B, the pump was drawing from an unmixed tote of hydrolysate which caused the back pressure valves for the C5 diaphragm pump to clog. The hydrolysate in the tote was particularly prone to causing clogs because it was squeezed using the small screw press which presses out more solids compared with the metso screw press.
    b. Resolution:
        i. A pvc pipe is now used to mix the tote well before pumping the hydrolysate to the propagators. The pipe is treated with hydrogen peroxide to sanitize prior to use.
    c. Status:
        i. Resolved.
12. Pretreatment acid pump
    a. Problem:
        i. One of the bolts holding the diaphragm together for the pretreatment acid pump broke off. This eventually led to neighboring bolts loosening, which then led to the diaphragm leaking a considerable amount of acid solution.
        ii. The pulse dampner was also leaking a considerable amount of acid solution.
    b. Resolution:
        i. The broken off bolt was removed and replaced.
        ii. The dampner fitting was welded to ensure no more leaking.
    c. Status:
        i. Resolved.