# Cellulose Production

1. Gluconoacetobacter xylinus was grown up on Heo and Son 0.1% glucose agar plates (using 12C-glucose) at 30oC without inositol (Heo and Son 2002).
2. Heo and Son liquid minimal media was made with 0.5% 0.1% glucose (make 1L as outlined in recipe).
3. Autoclave media and 10 foil covered 1L flasks
4. To increase surface area for cellulose growth, 100mL of the media was sterilely added to individual 1L Erlenmeyer flasks.
5. Each aliquot of media was inoculated with three isolated colonies of Gluconoacetobacter xylinus from Heo and Son plate.
6. Keep flasks static in the dark at 30oC for 2-3 weeks until thick cellulose pellicule forms.

# Cellulose Harvest

1. Pour off excess culture from cellulose growth flask
2. Make 1% Alconox solution
3. Add Alconox 2:1 with cellulose+ residual media (i.e. 200mL of 1% alconox for every 100mL media culture)
4. Autoclave for 30 minutes
5. Rinse under high pressure DI water faucet (~10 times, or until no suds being produced).
   * Use screen to hold over beaker to pour out water each time.
6. At end of rinsing, fill beaker with DI H2O and cover.
7. Let sit for 12 hours at room temp, then rinse each beaker 3 times under high pressure DI.
8. Fill with water and let sit for another 12 hours. Repeat this for 2 days (i.e. ~4 rinsings total)
9. On the third day (after the 4th 12 hour soak), rinse cellulose about 3 times, then decant all liquids and put only the cellulose on a pre-weighed weighboat.
10. Cover weighboat with another weight to prevent contamination (especially of streptomyces spores)
11. Place in drying oven overnight
12. Weigh dried pellicules and calculate difference (from weighboat)

## References

1. Moon-Soo Heo and Hong-Joo Son, Biotechnol Appl Biochem (2002) 36: 41-45