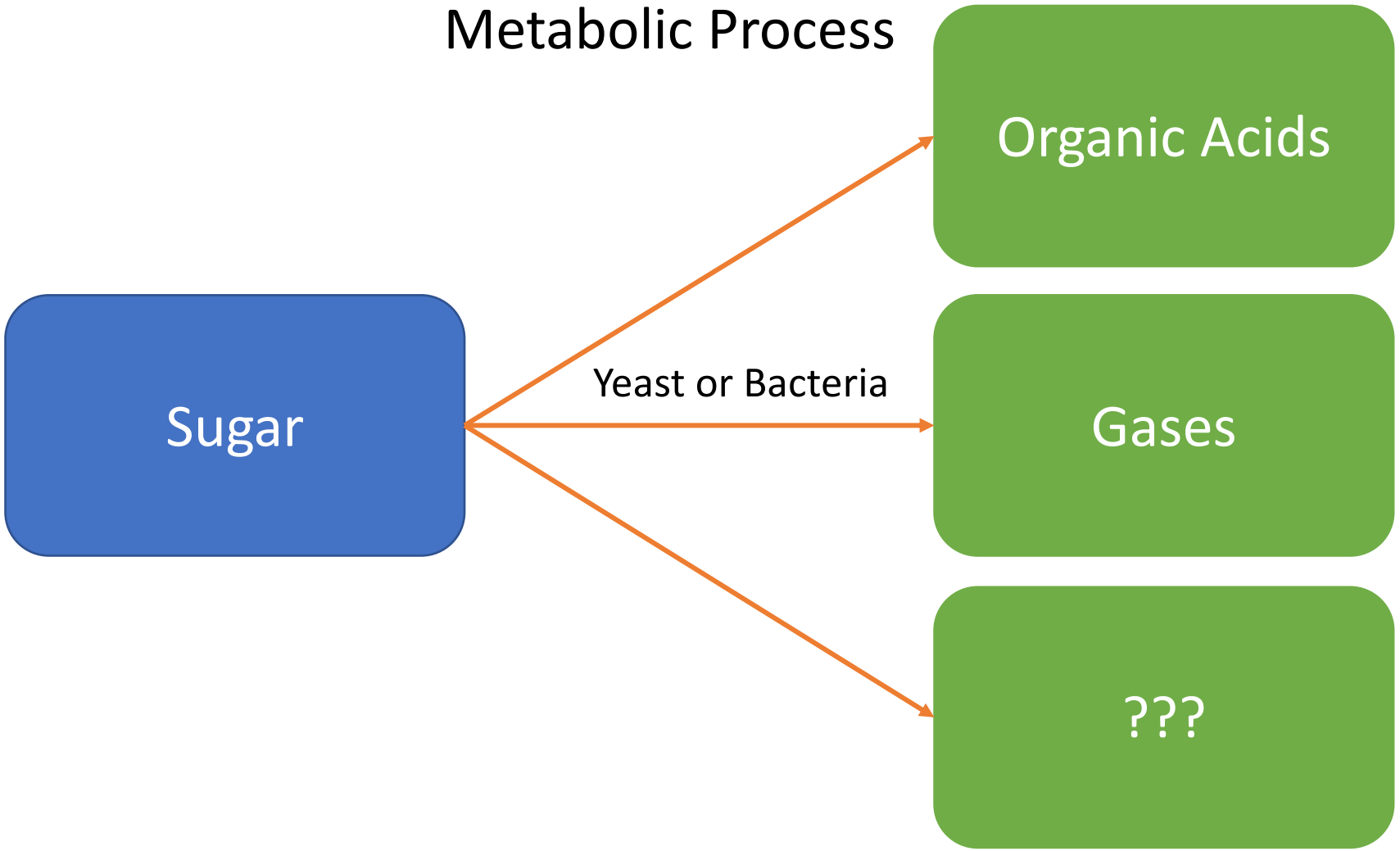
Fermentation Lab Presentation

ABE 304

Peer Evaluations

- 4 peer assessments throughout the semester
  - **Open February 6 - February 12 (42% response rate)**
  - Open February 24 – February 28
  - Open March 23 – March 28
  - Open April 27 – May 2
- Administered through CATME

Fermentation



Fermentation Products

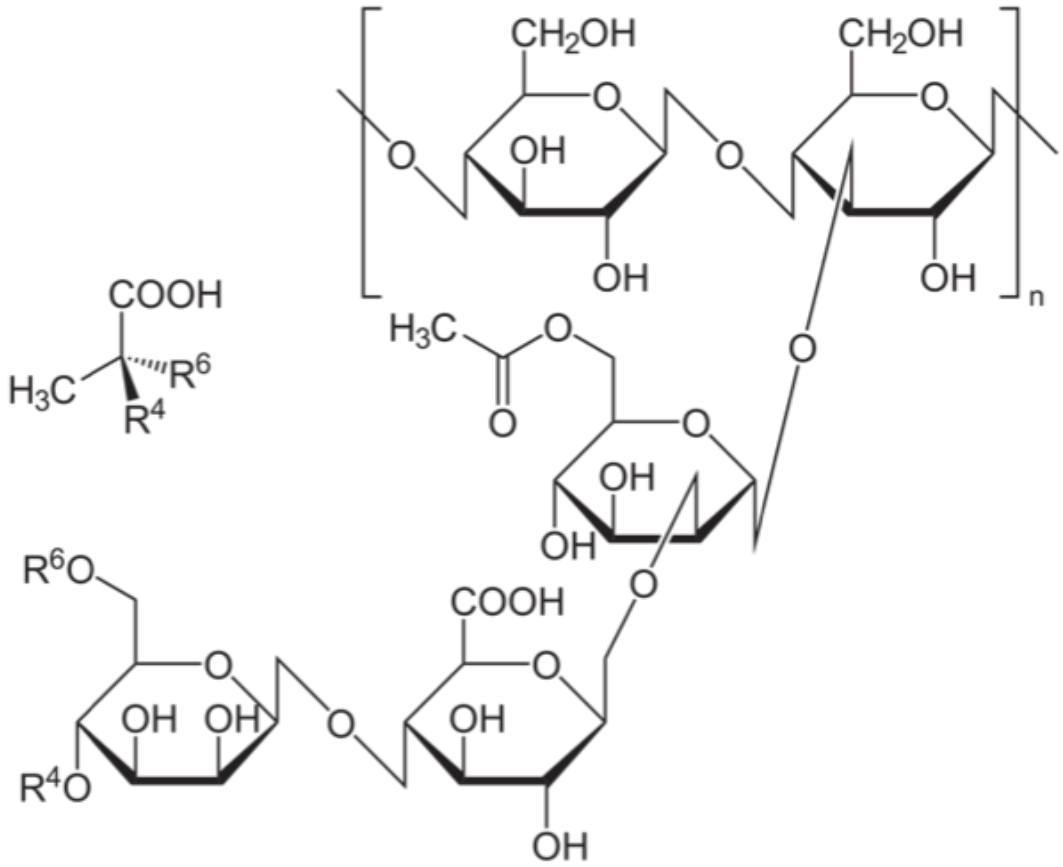
- How many fermentation products can you think of?

Fermentation Products

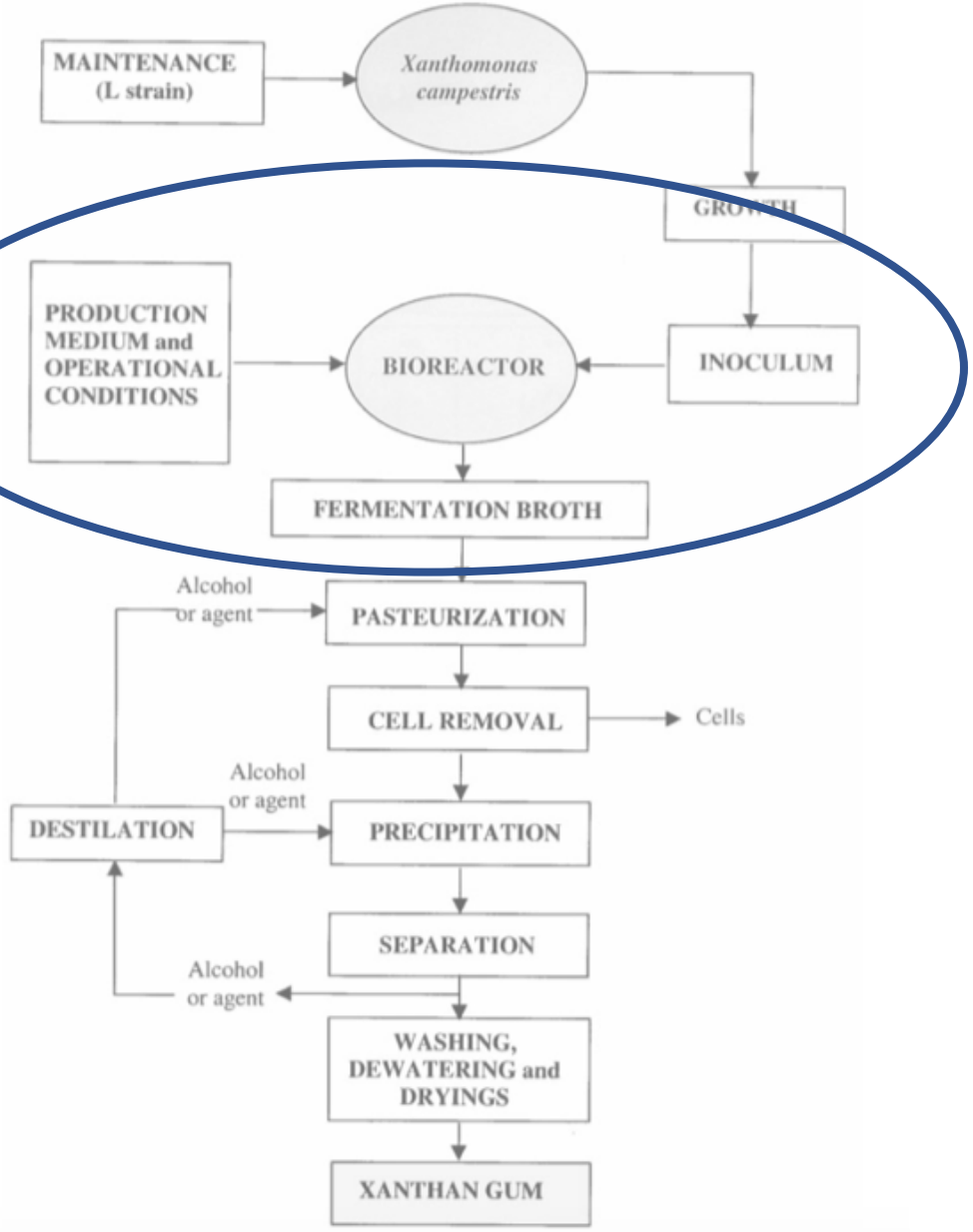
- Ethanol
  - Beer
  - Wine
  - Liquor
- Yogurt
- Kombucha
- Kefir
- Sauerkraut
- Kimchi
- Pickles
- Cheese
- Salami
- Prosciutto
- Sourdough bread
- Pharmaceutical products
  - Penicillin
  - Insulin
- Citric Acid
- Acetic Acid

Xanthan Gum

- Microbial Polysaccharide
- Produced from *Xanthamonas campestris*
- Significant rheological properties
- Cannot be synthesized



Industrial Production



Industrial Fermentation

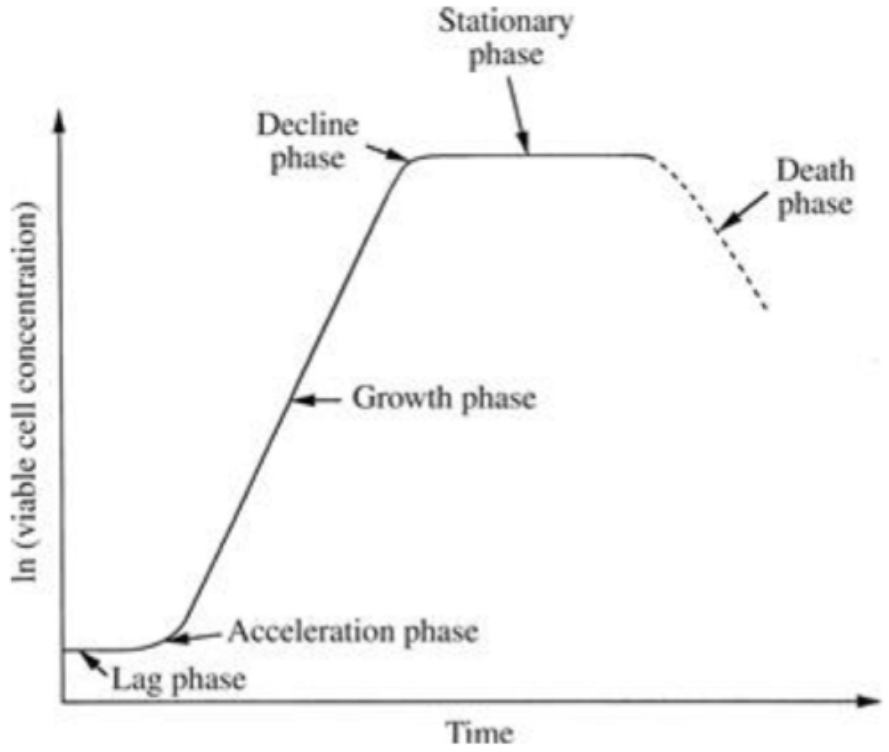
- Temperature uniformity
- Reactor size
- Internal pressure
- pH
- Oxygen distribution
- Nutrient availability
- Level of agitation

In the Lab

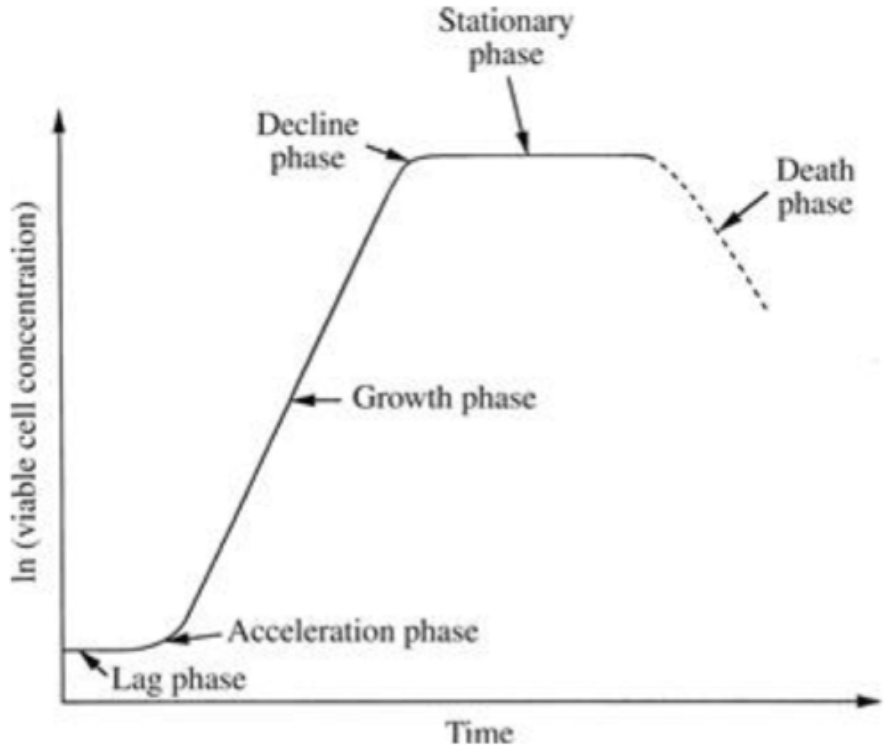
- Study effect of temperature on cell growth and xanthan production

25˚C	30˚C
Groups 1 & 2	Groups 3 & 4

Growth Kinetics



Growth Kinetics



$$r_x = \mu x \qquad r_x = \frac{dx}{dt}$$

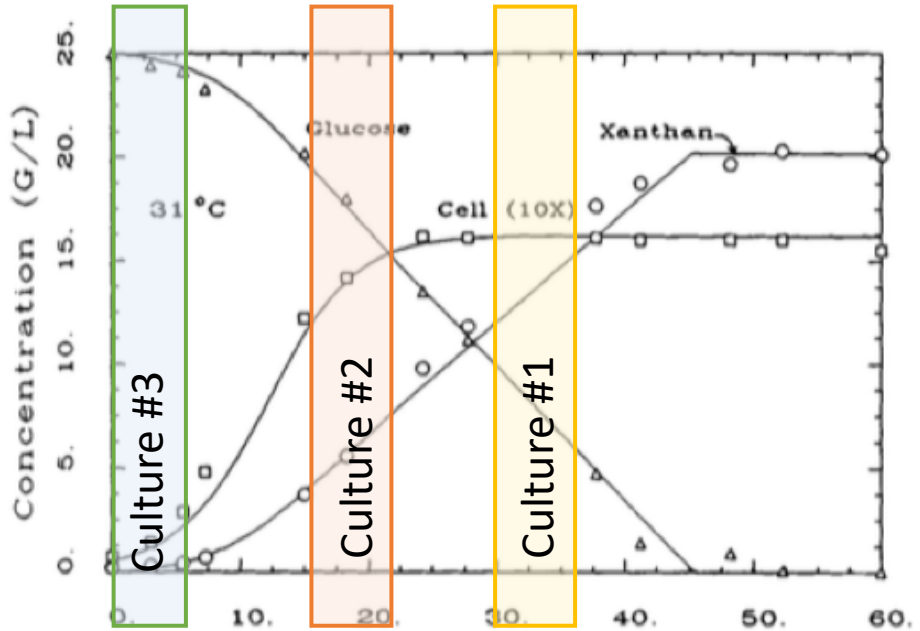
$$\frac{dx}{dt} = \mu x$$

$$\ln x = \ln x_0 + \mu\, t$$

$$x = x_0 e^{\mu t}$$

Lab – Week 1

1. Inoculate fermentation medium with *Xanthamonas campestris*
2. Take samples every 45 minutes for a total of 4 samples
3. Take samples from previously inoculated media to capture all phases of growth (12 samples total)



Cultures

- Each group will have three cultures
  - First—Two days prior to lab (TA) → 4 samples
  - Second—One day prior to lab (TA) → 4 samples
  - Third –Inoculate *Xanthamonas campestris* in class (Group) → 4 samples

5mL inoculum → 250 mL flask (with 100mL media)

Incubate your flask in the shaker at 25°C or 30°C (~150rpm)

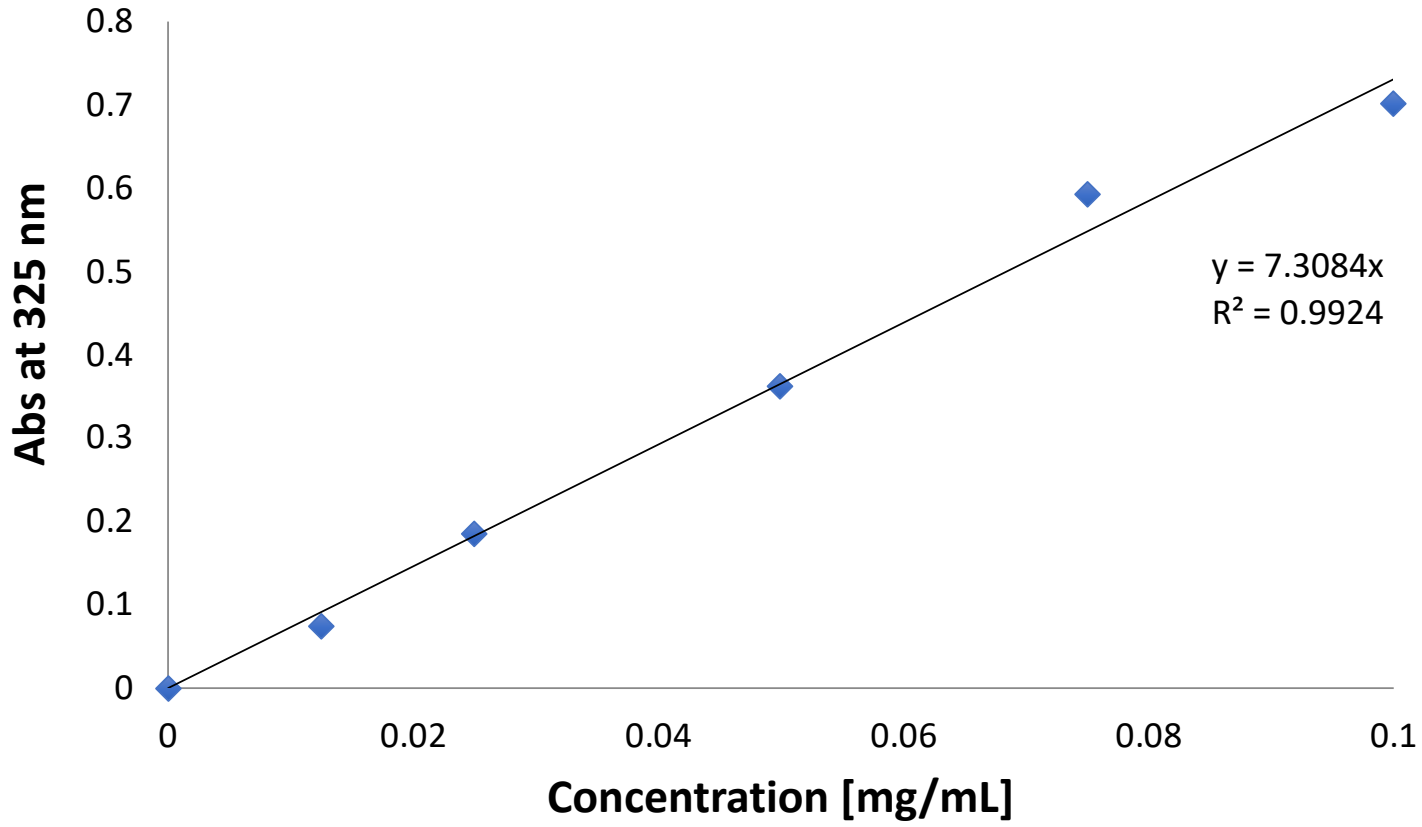
Yield

- Measure of how much of the substrate is converted into product

- Allows us to better account for
  - Reaction efficiency
  - Side Reactions

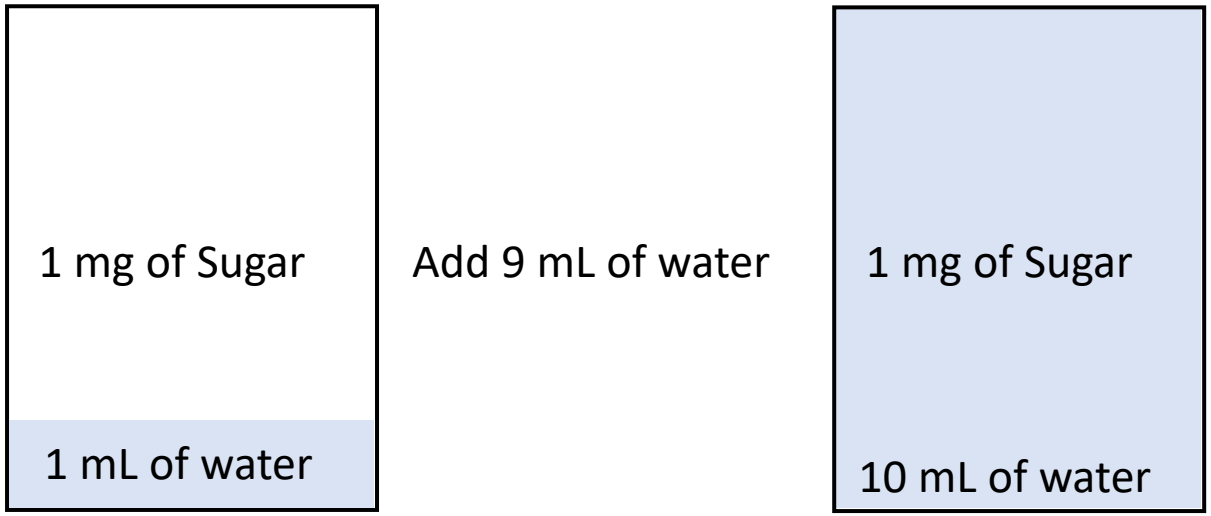
Calibration

Each spectrophotometer must be calibrated to be able to report concentration



Dilution

Some samples will need to be diluted to obtain accurate results



= 1 mg/mL

= 0.1 mg/mL

How diluted is the sample?

Lab – Week 1

Cell Growth

- [ ] Take sample
- [ ] Cell density (Spectrophotometer)
- [ ] Centrifuge
- [ ] Remove supernatant
- [ ] Measure with Spectrophotometer (at 600 nm)

  Blank: Media before inoculation

Supernatant

- [ ] Save supernatant in freezer for week 2

Sample

- 1 mL broth before inoculation (reference blank)
- 1 mL sample for Cell Density
- 1 mL sample x 2 = 2 supernatant sample for Glucose & Xanthan concentrations
  - Supernatant will be frozen for use during week 2

Total volume of each sample = 3mL

Lab – Week 2

Glucose Concentration

- [ ] Use frozen supernatant and dilute with water
- [ ] Add glucose oxidase (GOPOD kit)
- [ ] Measure with spectrophotometer (at 510 nm)
  Blank: GOPOD kit solution

Xanthan Concentration

- [ ] Xanthan calibration curve (Xanthan stock solution)
- [ ] Use frozen supernatant and dilute with water
- [ ] Sulfuric Acid digestion
- [ ] Measure with spectrophotometer (at 325 nm)
  Blank: Water