**Creating and Adding a Citrate Buffer**

Cellulase enzymes work by taking cellulose and converting it to glucose. This process occurs best in a solution with a pH of approximately 5.5 (+/- 0.5). One way to optimize enzyme activity is to add a buffer to the reaction to protect against pH fluctuation during incubation that can be affected by different biomass sources. Adding buffer is optional and can serve as another variable to test in this lab sequence. All sample data and graphs included in teacher materials were generated from data collected following the lab protocols without buffer.

0.5M citrate buffer works well for hydrolysis in CB2E. It can be prepared as follows:

In 100mL of distilled water, add:

* + 3.36 g citric acid, monohydrate
  + 10.00 g sodium citrate, dihydrate

Add 2.5 mL of citrate buffer to each test tube with 22.5 mL water and the 1g of biomass. Shake gently to mix up. Measure pH to confirm.