May 06, 2019

Working

A simple, accurate, low cost method for starch quantification in green microalgae

Tze Ching Yong[1], Chia-Sheng Chiu[1], Ching-Nen Nathan Chen[1]

[1]National Sun Yat-Sen University

dx.doi.org/10.17504/protocols.io.2mhgc36

Nathan Chen
National Sun Yat-Sen University

1 Harvest microalgal cells from 10 mL culture using swing bucket centrifugation (2600 *g* for 3 min).

2 Transfer the cells to a 2-mL screw cap microtube. Spin briefly and get rid of the medium using a pippet. Freeze the cells immediately at -15 °C in an ice-crude sea salt mix.

3 Add 1 mL methanol/tetrahydrofuran mix (v/v =1/3) to the cell pellet to extract pigments. Shake the microtube occasionally. Extract the pigments for at least 30 min.

4 Centrifuge at 16,000 *g* at 4 °C for 10 min. Discard the supernatant. Repeat the pigment extraction until the pellet becomes white, then dry the pellet at 65 °C in an oven for one hour.

5 Wash the pellet out of the microtube repeatedly using 50 mM, pH 5 sodium phosphate buffer, 5 mL in total, and transfer to a 15-mL tube.

6 Autoclave the cell suspension at 134 °C for one hour to disintegrate the starch granules.

7 Mix well and transfer 1 mL of the autoclaved sample to a 2-mL microtube, add 0.5 g acid-washed glass beads and then use a mini-beadbeater (BioSpec Products, USA) to smash the cells and release the starch (three cycles at the highest speed).

8 Transfer 0.5 mL of the beated sample to a microtube, add 0.5 mL sodium phosphate buffer (50 mM, pH 5) and 2 units (in 20 µL) of glucoamylase(1) for overnight digestion at 50 °C. Add the same amount of glucoamylase again the next morning for the second digestion in the same conditions for 7 hours.

9 After the double digestions, centrifuge the sample at 16,000 *g* at room temperature for 10 min and measure the glucose in the sample using the glucose assay protocol (the DNS method, see below).

Glucose assay using the DNS method

10 Build the standard curve for glucose assay: Prepare 10 mM glucose solution in 50 mM sodium phosphate buffer, pH 5.0, and do 2-fold serial dilutions until 312 µM using the buffer.

11 Add 0.5 mL the glucose solution (or a sample) to 2 mL of the DNS reagent(2) in a test tube (need to include 2 blanks to set zero). Mix well then heat the mixture in boiling water for 5 min. Cool down in tap water and measure the OD_{540} of the glucose standards and the samples.

12 Build the standard curve of OD_{540} against glucose quantities. Use the regression equation to calculate the glucose quantities in the samples.

Recipes and notes

13 (1) **Glucoamylase (TCI Chemicals, Tokyo, Japan; Cat. # M0035) prep**. : Take 100 units enzyme and dissolve it in 1 mL of 50 mM sodium phosphate buffer, pH 5 (17 mg of the enzyme powder contains about 100 units enzyme; the enzyme powder contains about 6000 units/g). This enzyme prep is good for 2 weeks if stored at 4 °C. Unit definition: amount of protein to cause degadation of 10 mg starch to glucose in 30 min (in specified conditions).

14 (2) **DNS reagent prep**: Dissolve 2 g NaOH in 70 mL H_2O, add 1 g of 3,5-dinitrosalicylic acid (DNS), then add 30 g potassium sodium tartrate. When the all chemicals are dissolved (takes about 2 - 3 days using sonication occasionally), bring the final volume to 100 mL. The final concentrations of the chemicals: 0.5 M NaOH, 44 mM dinitrosalicylicacid, 1 M potassium sodium tartrate.

15 (3) This protocol is based on the manuscript “Optimization of starch quantificationin green microalgae: a simple, accurate, low cost method using glucoamylase and dinitrosalicylic acid” submitted to PLOS ONE.