Dunaliella Cultivation

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Determination of glycerol

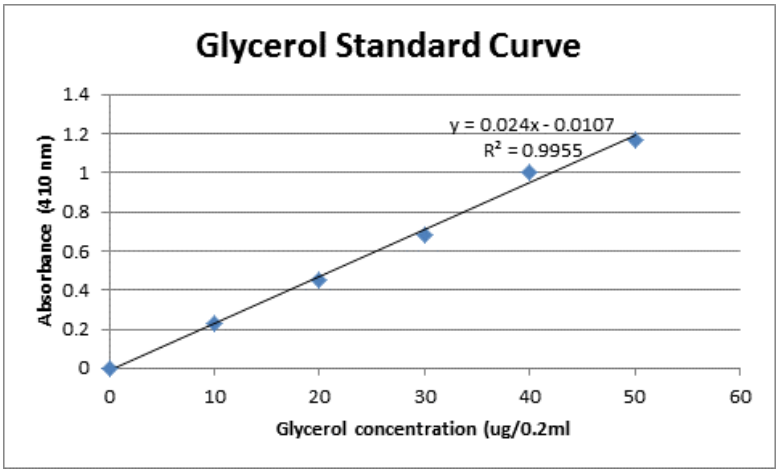
There are two methods to measure glycerol, a chemical and an enzymatic method.

Chemical Method

Chen et al., (2011).

- Harvest the cells by centrifugation at 5,000 g for 15 min at room temperature.
- Decant the supernatant and re-suspend the algal pellet by centrifugation in 1.0 mL of distilled water and 0.2 mL of chloroform.
- Sonicate the suspension for 8 minutes in an ultrasonic cell disruptor at room temperature.
- Remove cell debris by centrifuging the suspension at 10,000g for 10 min at room temperature and collect the supernatant.
- Take 100 µl of supernatant and make up to 0.2 mL with distilled water in a test tube.
- Add 1 mL of sodium periodate reagent (3 mmol/L sodium periodate and 100 mmol/L ammonium acetate in 100 mL 6% acetic acid) with mixing.
- After 5 minutes add 2.5 mL of acetyl acetone reagent (acetyl acetone/isopropanol=1:99) with mixing.
- Place the test tube in a water bath at 60 °C for 30 min. Place a marble at the mouth of the tube to restrict evaporative loss during incubation.
- Cool to room temperature then read the absorbance at 410 nm. The corresponding concentrations of blank samples without algae should be run in each test.

The absorbance of each sample can be converted to glycerol content by reference to a standard curve for pure glycerol (0-50 µg glycerol /0.2 ml) .



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Enzymatic method

Microalgal extract preparation

After Ben-Amotz and Avron (1973).

- Harvest the algal cells by centrifugation at 5,000 g for 15 min at room temperature.
- Decant the supernatant and re-suspend in 1.5 mL of distilled water.
- Sonicate the suspension for 8 minutes in an ultrasonic cell disruptor at room temperature.
- Remove cell debris by centrifuging the suspension at 10,000g -12000g for 10 min at room temperature and collect the supernatant.
- Deproteinize the suspension by adding trichloroacetic acid to 2%w/v final concentration, then neutralize with NaHCO.

Enzymic Determination of glycerol

The glycerol content in the algal cells is determined by enzyme-coupled assay in which glycerol is oxidised to dihydroxyacetone and NAD^+ reduced to NADH using glycerol dehyrogenase in the following reaction:

Glycerol + NAD ⇔dihydroxyacetone + NADH

One unit of glycerol dehydrogenase reduces one micromole of NAD per minute at 25°C and pH 10.0 under the specified conditions.

Pipette into a 1ml UV cuvette the following:

- algal extract
- 0.1M NAD
- make up to 1 ml with 0.125 M carbonate/bicarbonate buffer, pH 10.0.

Start the reaction with the addition of 10 µl of glycerol dehydrogenase (100unit/ml in 0.05 M potassium phosphate buffer solution).

Determine the increase in absorbance at 340 nm resulting from the reduction of NAD. The concentration of glycerol can be calculated using the extinction coefficient for NADH (ε340 = 6220 M-1cm-1)

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