

Quantitative Analysis of Chlorophyll-a from Pacific Sea Plasma

Background:

Spirulina contains only Chlorophyll-a. Carotenoids and chlorophyll are acetone soluble but the carotenoids do not interfere in this spectrophotometric method as the absorbency for carotenoids range from 400-500 nm, and the absorbency for chlorophyll A is 666 nm.

The other major pigments in *Spirulina* are the water-soluble phycocyanins, these remain in an acetone-insoluble pellet during the assay.

Equipment and instruments:

Analytical balance

Centrifuge

Drying pans

Drying oven at 110 degrees C.

85 % acetone in water

Spectrophotometer

35 ml round bottom glass centrifuge tubes with caps

50 ml volumetric flask with lid

Vortexer (Maxi Mix II)

Centrifuge

Glass beads (through 20 mesh)

Pipettes

Method:

Dry Weight

- 1) Place drying pans in oven for 30 minutes place in desiccator to remove excess moisture.
- 2) When pans are cool, weigh and record weight of pan.
- 3) Tare the balance with the pan on it and place about two grams of powder in the pan.
Record the weight of the powder.
- 4) Place pan and powder in the oven and dry for two hours.
- 5) Remove pan and powder from the oven and place in desiccator 15 minutes to cool.
- 6) Weigh and record the total weight of the pan and the dry powder.
- 7) Perform duplicates for each sample.

Chlorophyll A Assay

- 1) Weigh approximately 50 mg of Spirulina into a 35 ml centrifuge tube. Record weight.
- 2) Add 5 grams of glass beads and 2.5 ml of 85 % acetone in water.
- 3) Vortex vigorously for 5 minutes.
- 4) Add 10 ml of 85% acetone in water, vortex briefly and centrifuge at 3200 RPM of 5 minutes.
- 5) Collect the supernate in a 50 ml volumetric flask .
- 6) Repeat steps 3-5 until supernate is clear. Four extractions should be sufficient.
- 7) Bring the flask up to volume with 85% acetone in water and cap the flask and invert gently to mix the contents.
- 8) Read the absorbency with the spectrophotometer at 666nm and 642 nm against an 85 % acetone/water blank.

Calculations:

Dry weight

$$\text{Percent dry wt} = \frac{(\text{pan (g)} + \text{dried powder (g)}) - \text{pan wt (g)}}{\text{powder wt (not dried) (g)}}$$

Chlorophyll A

$$\text{Chlorophyll A (\%)} = \frac{[(9.93 \times \text{Abs}_{666}) - (0.0777 \times \text{Abs}_{642})] \times 0.05 \text{ liter} \times 100}{\text{Sample weight (mg)} \times \% \text{ dry wt.}}$$

References

A.O.A.C. Official Methods of Analysis (1995); 940.03

Ind. Eng. Chem. Anal. Ed. 12:148 (1940) and 15:524 (1943).