Pn°3. Determination of photosynthetic pigments

1. Introduction

Photosynthesis is a biochemical mechanism that enables higher plants to produce carbohydrates from water and CO2, this reaction requires an external supply of energy in the form of light, this reaction takes place in organelles specific to the chlorophyll cell: chloroplasts.

Many proteins and other molecules contribute to the mechanism including photosynthetic pigments. Plants have three types of photosynthetic pigments: chlorophyll (a and b) and carotenoids, which are present in all carbon autotrophic plants and phycobilin which are found only in algae and cyanobacteria. Lipophilic molecules specifically absorb light at specific wavelengths.

2. Objective

Extracting and measuring chlorophyll a, b and carotenoids from spinach leaves using absorption spectrometry.

3. Materials and chemicals

- Mortar and pestle
- Test tube (25, 50, 100 ml)
- Filter paper
- Beaker (25, 50 ml)
- Erlenmeyers (25, 50 ml)
- Small funnel
- Pasteur pipette
- Pipette (1, 5 et 10 ml)
- Acetone solvent 80%
- Spectrometer

4. Methodology

a. Extraction of photosynthetic pigments

- Wach, dry and weigh 1.5g spinach leaves.
- Cut plant material into small fragments.
- Add 4 ml acetone and grind to homogeneous mixture.
- Add 6 ml acetone and grind again.
- Allow to settle for 10 min
- Collect the supernatant in a 10 ml Erlenmeyer flask (Filter on filter paper and collect the chlorophyll solution).
- Close with parafilm and shake.

b. Determination of total pigments:

- Add 2 ml of pigment extract to a 20 ml volumetric flask and complete with acetone.
- Seal the vial with parafilm and shake.
- place a glass vial containing the extraction solvent in the beam and zero the instrument at 460 nm.
- Replace this vial with another containing 2/3 of the diluted pigment extract and read the absorbance A.

- Repeat the operations successively at 645 and 663 nm (set the instrument to zero for each wavelength used).

5. Results:

The formulas below are based on BEER-LAMBERT's law for calculating pigment concentrations.

Ca = 12.7 A663 - 2.63 A645
Cb = 22.9 A645 - 4.68 A663 in mg/l
Ccar = 5 A460 - (3.19**Ca** +130.3**Cb**)/200