

## 1. Procedure summary

Procedure for obtaining the dry weight of harvest sample expressed in g/L. These samples are prepared and then dried in an oven.

### 1.1. Related Procedures

Oven Dry weights GL-07-003-002

### 1.2. Procedure impacts and concerns

Safety	Safety glasses should be worn at all times while performing this procedure. Heat resistant gloves are required when removing samples from the oven due to high oven temperatures. Latex gloves should be worn as needed.
Quality	All samples are run in triplicate and should be within 10% RSD of each other or less. Weather events or debris could possibly causes excessive higher %RSD for triplicate values.
Delivery	Samples must be analyzed and all data entered into L:\QAQC\Raw Data\Cultivation\Dry Weights- Valve or L:\QAQC\Raw Data\Harvest\Dry Weights by Saturday at noon of harvest week.
Environmental	Local policies and procedures should be followed as determined by the site leadership.
Cost	Whatman filters are \$1.03 per filter, Wyvern are \$0.59 per filter.
Compliance	Compliance with OSHA's Hazardous Waste Operations and Response, and Hazardous Communication Standard in addition to the Sapphire Energy, Inc. Chemical Hygiene Plan is required (see 29 CFR 1910.120 and 1200).

### 1.3. Responsibilities and owners

Document Owner	Manage content and distribution	Kari Mickelson
Process Owner	Responsible for content and process validation	Tonia Lane
Plant Manager	Responsible for implementation and conformance	Rebecca White

## 2. Process

### 2.1. 2.1 Process description

Dry weights are a measure of the total suspended solids (TSS) within a sample. Typically there are 7 types of Samples taken during an IABR harvest. Samples include but are not limited to AH-V202, AH-V402, DFP, SN, Centrate, TA and Slurry. Two methods that can be performed on Harvest Dry weights, a Volumetric weight using GF/C (47mm) filters and a Gravimetric weight using GF/D (90mm) filters. A volumetric measurement which consists of filtering a known volume of sample using a pre weighed filter paper that will retain solids larger than the pore size of the filter. Gravimetric measurements weight consisting of filtering a known weight of a sample through a pre weighed filter paper that will retain solids larger that pore size of filter. In both process methods after filtering has occurred the filter paper is dried to remove all moisture, allowed to cool and then weighed. The increased weight of the filter paper represents the total suspended solids in the sample. These measurements are used to determine harvest productivity.

### 2.2. Process diagram:

Not applicable for this process.

### Equipment and Supplies

- GF/C (47mm) filters (Whatman #1822-047) (Wyvern #C4700)
- GF/D Filters (90mm) (Whatman #.....)
- Squirt bottle filled with pure water (nano or DI)
- Disposable aluminum weigh dishes (Fisher #08-732-102) and (Fisher #.....)
- Forceps
- 25mL Serological pipets
- Pipet Aid
- Squirt bottle filled with 2 pptousand ammonium bicarbonate
- Heat shielding gloves
- Oven trays
- Magnetic Filter Funnel 47mm system
- Buchner Funnel 90mm system
- Analytical Balance
- Yamato DX600 Drying Oven
- Desiccator
- Mesh Sink Trap

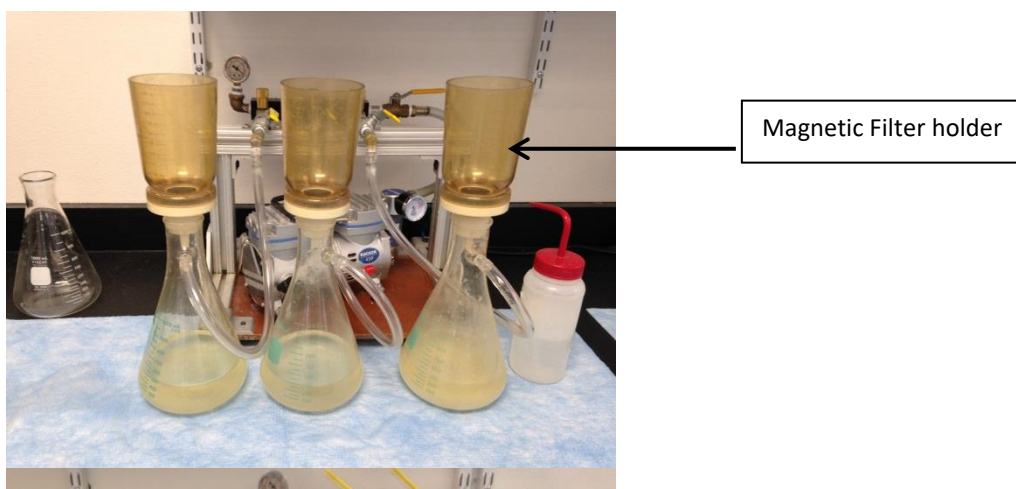


Figure 1.1 Magnetic Filter Funnel Apparatus for GF/C (47mm)

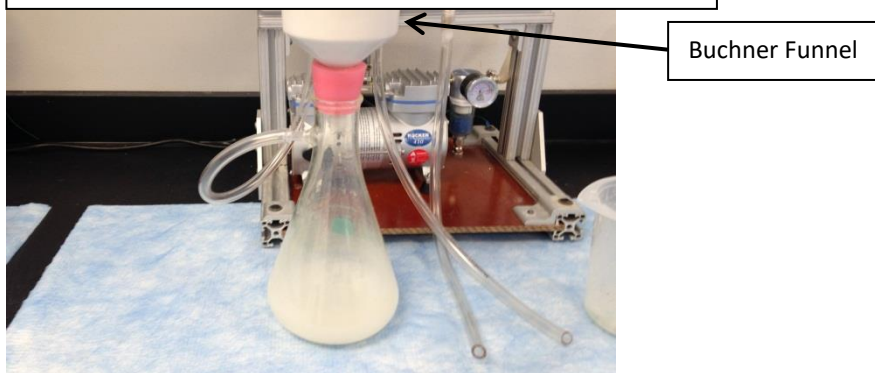


Figure 1.2 Buchner Funnel Apparatus for GF/D (90mm) Filters

**2.3. 2.3 Process step****2.3.1 Filter preparation**

- 2.3.1.2 Place appropriate sized aluminum weigh dishes on oven trays.  
Remember each sample will be run in triplicate.
- 2.3.1.3 Set up the Magnetic Filter Funnel apparatus without the magnetic filter holder for GF/C (47mm) filters see Figure 1.1. Setup Buchner Funnel Apparatus from Figure 1.2 for GF/D (90mm) filters.
- 2.3.1.4 Place new GF/C (47mm) filter on the filter screen. See Figure 1.1. for GF/C (47mm) filters. If using GF/D (90mm) filters place new GF/D (90mm) filter in Buchner Funnel. See figure 1.2 for GF/D (90mm) filters.
- 2.3.1.5 Apply vacuum (up to 100 kPa) and rinse the filter with a generous amount of DI water or nano water.
- 2.3.1.6 Remove the filter with forceps and place it in the aluminum weigh dish.
- 2.3.1.7 Place trays in the oven at 105°C to dry for at least 1 hour.
- 2.3.1.8 Transfer the trays of dried filters to the desiccator to cool using heat shielding gloves.
- 2.3.1.9 Allow the trays to cool for a minimum of 10 minutes.
- 2.3.1.10 Print updated Harvest Dry Weight Data Template located in L:\QAQC\Data Analysis Templates. Use either Harvest Dry Weight Data Template (G) or Harvest Dry Weight Data Template (V) depending on samples.
- 2.3.1.11 Number each aluminum weigh dish tray.
- 2.3.1.12 Weigh each dish using an analytical balance.
- 2.3.1.13 Record weighed values under Tray+Filter for each tray on printed Harvest Dry Weight Data Template. Number on aluminum tray should correspond to same number on printed Harvest Dry Weight Data sheet

**2.3.2 Filtering samples using GF/C (47mm) filters (Volumetric)**

- 2.3.2.1 Typically GF/C (47mm) filters are used for AH-V202, AH-V402 DFP and SN samples.
- 2.3.2.2 Set up the Magnetic Filter Funnel apparatus without the magnetic filter holder. (See Figure 1.1)
- 2.3.2.3 Using forceps, place a prepared GF/C (47mm) filter from process 2.3.1 on the filter screen.
- 2.3.2.4 Place the magnetic filter holder on top of the filter.
- 2.3.2.5 IABR samples require a mesh on top of the funnel head to act as a pre-filter for the samples. This helps with data quality in cases where the sample is contaminated with excessive silt and or non-algal organic material (e.g. plant and bug matter).
- 2.3.2.6 Mix samples by inverting several times.
- 2.3.2.7 Using a serological pipet dispense 25mL of culture into the magnetic filter holder. Volume may vary. See QAQC supervisor for any questions.
- 2.3.2.8 Turn on the vacuum and allow liquid to flow through the filter. Please note that if the system is air tight then the vacuum pump can be turned off once a vacuum is established.

- 2.3.2.9 Rinse the sides of the cup and the filter with 2ppthousand ammonium bicarbonate ( $\text{NH}_4\text{HCO}_3$ ) using a squirt bottle.
- 2.3.2.10 Remove the magnetic filter holder and mesh trap and place the filter back in the same aluminum weigh dish using forceps.
- 2.3.3 Filtering samples using GF/D (90mm) filters (Gravimetric)
  - 2.3.3.1 Typically GF/D (90mm) filters are used for Centrate, TA and Slurry samples.
  - 2.3.3.2 Setup Buchner Funnel Apparatus seen in Figure 1.2.
  - 2.3.3.3 Using analytical balance weigh out .7-1g of each sample. When weighing samples use new aluminum weigh dishes. Remember samples are done in triplicate.
  - 2.3.3.4 Record weighed values under Sample Weight (g) on printed Harvest Dry Weight Data Template.
  - 2.3.3.4 Using forceps, place a prepared GF/D (90mm) filter from process 2.3.1 in Buchner filter screen. See Figure 1.2
  - 2.3.3.5 Re-suspend weighed out samples from 2.3.3.3 in 2ppthousand ammonium bicarbonate ( $\text{NH}_4\text{HCO}_3$ ).
  - 2.3.3.6 Once samples are re-suspended turn on vacuum and dispense re-suspended samples on GF/D (90mm) filters. Allow liquid to flow through filter.
  - 2.3.3.7 Rinse the sides of Buchner funnel and filter with 2ppthousand ammonium bicarbonate ( $\text{NH}_4\text{HCO}_3$ ) using a squirt bottle.
  - 2.3.3.8 After liquid has flowed through filter place filter back in same aluminum weigh dish as it was in before filtration.
- 2.3.4 Drying of Samples
  - 2.3.4.1 When all samples have been filtered place trays in the oven to dry for at least 1 hour. (Check that the oven is set at 105°C)
  - 2.3.4.2 Transfer trays of dried filters to the desiccator using heat shielding gloves.
  - 2.3.4.3 Allow trays to cool for a minimum of 10 minutes.
  - 2.3.4.4 Weigh each dish using an analytical balance.
  - 2.3.4.5 Record these values under Tray+Dry Weight on printed Harvest Dry Weight Data Template sheet. Number on aluminum tray should correspond to same number on printed Harvest Dry Weight Data sheet
- 2.3.5. Dry weight Data entry into L:\ drive.
  - 2.3.5.1 Harvest Dry Weight Data Template can be located L:\QAQC\Data Analysis Templates.
  - 2.3.5.2 Open up either Harvest Dry Weight Data Template (G) or Harvest Dry Weight Data Template (V) depending on samples.
  - 2.3.5.3 Immediately after opening file go to "Save as" and save file in L:\QAQC\Raw Data\Harvest\Dry Weights. (e.g. 20141115 Harvest Dry Weights).
  - 2.3.5.4 Enter all data record from printed Harvest Dry Weight Data sheet into analysis template. Excel will automatically calculate dry weight in g/L. If RSD% is over 10% dry weights need to be ran again and reported to supervisor.
  - 2.3.5.5 Dry weight calculations in excel

2.3.5.1 Calculate the dry weight in g/L using GF/C (47mm).

Dry Weight=((Tray+Dry Weight – Tray+Filter  
Weight)/25mL)\*1000)

2.3.5.2 Calculate the dry weight in g/L using GF/C (90mm).

Dry Weight=((Tray+Dry Weight – Tray+Filter  
Weight)/Sample weight)\*1000)

**2.3.6 Preparation of Reagents & Solutions**

**2.3.6.1 Ammonium Bicarbonate  $\text{NH}_4\text{HCO}_3$  (2ppt)**

Ammonium Bicarbonate ( $\text{NH}_4\text{HCO}_3$ )	4 g
DI water	Dilute to 2 L

2.3.6.2 Dissolve 4.0 g ammonium bicarbonate in about 1.5 L

DI water in a 2 L beaker or flask using stir plate.

2.3.6.3 Mix until reagent is completely dissolved.

2.3.6.4 Dilute to 2 L using DI water. Mix again using stir  
plate.

**3. Required documents**

**3.1. Input documents**

Harvest Sample sheet

Harvest Sampling sheet MASTER

**3.2. Output documents**

Excel or OSI-PI data spreadsheet

Harvest Dry weight Data

**4. Document control**

**4.1. Revision history**

Initial Release – Miguel Montoya	01/06/2014

**4.2. Document approval**

**4.3. Document reviewers**

**5. Risk analysis**