

## Appendix A:

Protocols for sample handling for  
“Global Database of Microbes in Anaerobic Digesters”

October 2, 2018

### Sampling Guide

We suggest that sampling takes place as a four-step process:

1. Coordinator selects the relevant anaerobic digesters and makes sure the operators have necessary sampling equipment and containers, as well as instructions about the sampling, storage and transport.
2. Sampling at the plant and transport to the coordinator.
3. Preservation of the samples and storage by the coordinator.
4. Shipment of the samples to Aalborg, Denmark by courier.

### Sampling from Anaerobic Digesters (step 2)

#### Investigate digester operation.

##### Key points:

Have the operation conditions been unstable the last couple of days prior to sampling (operation stops, foaming, excessive use of chemicals or other irregularities, which may impact the analysis)?

Major deviations should be reported in Appendix B, provided to all country coordinators.

##### Solids (SS or TS):

Approximate content of solids (SS or TS in g/L) for the digesters should also be reported to the country coordinator. **This information is extremely important to ensure proper handling during DNA and RNA preparation, and must be supplied alongside shipment of samples.**

#### Sampling.

Two independent samples should be drawn separately from each digester, and treated as individual replicates (i.e. not mixed together). Samples should be collected **during mixing** if possible, to ensure that the sludge is sufficiently homogenous.

- Remove sludge from dead volumes: Open valves and discard sludge stagnant in pipes and hoses, which does not reflect mixed reactor conditions (**remove at least 1 L**).
- Sample A: Open valve again, and collect sludge in a bucket (approximately 1 L). Transfer sludge to a suitable container, and fill the container as much as possible to minimize the oxygen.
- Sample B: Open valve again, and collect sludge in a bucket (approximately 1 L). Transfer sludge to a suitable container, similar to sample A.

NB: Keep the two samples separate in individual containers.

- Close sampling containers tightly and store on ice/in a fridge.
- Remember to properly label the samples (date, content, plant of origin, and supply SS/TS values to coordinators).

#### **Storage and shipment to coordinator**

- Store sample on ice or in the fridge at all times (do not freeze!).
- Send/deliver the sample to the country coordinator the same day as sampling (delivery should ideally be completed no later than the following day). If possible, send the samples on ice or with cooling elements.

### **Coordinator handling of samples, storage and shipment (step 3)**

#### **Receiving samples from Anaerobic Digesters**

We recommend that handling of samples is performed as soon as the samples arrive from the sampling sites. If this is not possible, then handling should be performed within the same day, with samples stored in the fridge prior to handling (do not freeze!).

In case handling can only be performed after a day, we kindly ask you to note this in the metadata sheet (Appendix B) under *sample notes*.

#### **Handling samples**

For each anaerobic digester two independent samples should be delivered to the coordinator. Keep the samples separate, and add the one sample to the A tube and the other sample to the corresponding B tube.

If there are cases where only one sample has been retrieved per digester, please split it into two tubes, and make a notation in the metadata sheet (column A: "Single sample split into tube A and B").

- Shake the container vigorously: Take 3 mL of sample (with the provided disposable pipettes). Add it to the provided 15 mL Grainer tube, which already contains 3 mL preserving liquid (RNAlater™).
- Lock the lid tightly and mix the sample gently by inverting it a couple of times.
- Repeat for the duplicate sample.
- Take note of the tube number, and be sure to fill out the metadata excel sheet (Appendix B) with sample and digester information.
- Place in a fridge.

*Note: The sample preservative (RNAlater™) does not contain any substances that are considered to be hazardous to health. In case of skin or eye contact rinse with water (no immediate medical attention is required). RNAlater™ is not expected to present ingestion or inhalation hazard under anticipated conditions of normal use. No special handling advices are necessary.*

#### **Storage**

Tubes containing preservative (RNAlater™) can be stored at ambient temperature until sample material is added. Once the sample material has been added, place duplicate samples in the fridge at all times, max. up to two weeks prior to shipment.

## Shipment (step 4)

*Prior to shipment: ensure that cooler elements have been frozen.*

- Repack the sample tubes in the provided biological substance boxes (as illustrated on the picture below)



1: Place up to 10 tubes into the bubble wrap pouch that also contains absorbent material. 2: Place the pouch into the safety bag and close. 3: Place the packed tubes into the biological substance box and close it.

- Place the biological substance boxes into a flamingo box and place cooler elements around the biological substance boxes (ensure that everything is tightly packed to minimize severe turbulence during shipment).
- Place the address label on the outside of flamingo box.

Address: Aalborg University  
Section of Biotechnology  
Att: Vibeke R. Jorgensen (phone: +45 2617 9672)  
Fredrik Bajers vej 7H  
DK-9220 Aalborg East  
Denmark

*We have supplied you with a label that has already been pre-filled with our address that you can attach to the package.*

- Arrange shipment with your preferred courier company. **VERY IMPORTANT: Choose priority shipment.** Notice that if you require us to pay for the shipment we can only accommodate FedEx or DHL (for European samples we generally prefer DHL shipments).

*NB: if you require us to pay for the shipment, please contact Vibeke (vrj@bio.aau.dk) ahead of time to get an account number*

- For samples sent from outside EU: please send packages in the **beginning of the week (Monday or Tuesday)** to ensure that the package is not stranded during the weekend.

Please notify Vibeke by email ([vrj@bio.aau.dk](mailto:vrj@bio.aau.dk)) when you have sent the samples, preferably with tracking number for the package. To ensure that we receive the metadata connected to all samples, we ask you to attach the completed metadata sheet (Appendix B) along with this email. **Samples will not be processed until we receive metadata for the samples.**