

Sampling Protocol Case 2: Send frozen pellets

In short, please i) complete the sampling data sheet, ii) take 3 mixed liquor samples from the **aerobic zone** of one aeration tank in a plant using activated sludge process^{*}, iii) centrifuge and store at -80°C/-20°C, iv) send back with dry ice.

Detail procedures

1. Before sampling:
 - (1) Prepare a cooler with enough ice (the provided foam box can serve as cooler), Sampling Data Sheet, 50-ml tubes (1 tube/sample), sampler, gloves, a wash bottle containing clean water, pen and marker pen (tools provided except ice and wash bottle).
 - (2) Fill the Sampling Data Sheet as fully as possible by communicating with the supervisor or manager of the wastewater treatment plant.
2. Sampling:
 - (3) Figure out sampling positions.
Typical sampling positions in an aeration tank are at the front, the middle and the end part of aerobic zone. If you can only reach one position of a tank, please take 3 sequential samples with 30-min interval.
 - (4) Wear gloves. Take out a 50-ml tube with proper label of this position (provided). You may write a new label if necessary.
 - (5) Rinse the sampler by mixed liquor at the position. Take at least 1 liter mixed liquor.
 - (6) Shake or stir this 1-L mixed liquor and then transfer 40~50 ml to the tube.
 - (7) Cap the tube tightly, chill on ice, and cap the cooler. Wash the sampler by clean water.
 - (8) Fill the sampling information in Sampling Data Sheet (provided).
 - (9) Go to next position and repeat step (4)~(8).
3. Centrifuge and storage
 - (10) In lab, shake up a sample, divide into 4 x 1.5ml and 1 x 13ml and discard the rest.
** Keep the tubes on ice to keep liquor samples cold.*
 - (11) Centrifuge at 4°C, 15,000 g for 10 min. Discard the supernatant.
 - (12) Preserve the pellets at -80°C (-20°C is applicable for a short-term storage).
4. Packing and shipping:
 - (13) Please plan to send samples to us on Monday or Tuesday or Wednesday.
 - (14) Prepare dry ice (4~5 pounds/package). Consult a courier for notes and requirements of shipping with dry ice. Schedule FedEx previously if necessary.
 - (15) When packing into the foam box, put dry ice and the samples as close together as possible with enough dry ice on top. Fill any empty space with wadded newspaper.
 - (16) Band the foam box and outer packaging tightly by tape. **DO NOT SEAL DRY ICE.**
 - (17) Paste “Dry Ice Label” and “Freeze Label” on the side.
 - (18) Send by FedEx using the “FedEx US Airbill” we provided (we will pay ☺).
Remind the courier it is an overnight shipping. Let us know by email or phone call.

Thank you very much! Please feel free to contact us if you have any questions.

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^{*} We may confirm the number of samples with you before sampling. Our objective is to collect at least 3 samples per plant and at least 12 samples per site (large city or region).

Optional centrifuge solutions in Case 2

- If your centrifuge cannot be set at 4°C, you need to keep mixed liquor samples at room temperature instead of chilling on ice, and centrifuge within 3 hours after sampling. If this is also difficult, you may change to our “Case1” protocol and sampling kit, and send the mixed liquor samples to us within 12 hours after sampling.
- If your centrifuge can deal with 1.5-ml tubes but not 15-ml tubes, you may divide each sample to 8 x 1.5ml.
- If your centrifuge can deal with 15-ml tubes but not 1.5-ml tubes, you may divide each sample to 3 x 3ml and 1 x 10 ml in 15-ml tubes.

#	Materials in the sampling kit we provide	Number
1	FedEx US Airbill for shipping to Oklahoma University	1
2	Dry Ice Label	1
3	Freeze Upon Arrival Label	2
4	Sampling Protocol	1
5	Sampling Data Sheet	_____ copies
6	Notes about process type in sampling data sheet	1
7	Example 1 of completed Sampling Data Sheet	1
8	Example 2 of completed Sampling Data Sheet	1
9	Labeled sampling tubes (50ml) in sealed plastic bags	_____ prep.
10	Additional sampling tubes	_____ prep.
11	Labeled centrifuge tubes in sealed plastic bags 4 of 1.5-ml tubes and 1 of 15-ml tube per prep.	_____ prep.
12	Additional centrifuge tubes 4 of 1.5-ml tubes and 1 of 15-ml tube per prep.	_____ prep.
13	Additional sealed plastic bags	_____
14	Marker pen	1
15	Pen	1
16	Gloves	_____ pairs
17	Foam box	1
18	Wadded newspaper	/

Other necessary tools not provided in this kit:

1. Centrifuges (for 1.5-ml tubes and 15-ml tubes)
2. Freezer (-80°C or -20°C, for pellet storage)
3. Ice (to keep mixed liquor samples cold)
4. Dry ice (for shipping frozen pellets to Oklahoma University)
5. Wash bottle (to wash the sampler)
6. Tape (for packing)
7. Sampler (a plant usually has samplers. If not, you may use a 1-L bottle with 15-foot rope as a sampler.)
8. Other protective supplies (the plant may require and provide, e.g. hard hat)