# Biomass measure

Several parameters of biomass state can be measured from culture's sample using different fixation/quenching techniques.

- *Measure of dry biomass residuals*

This is a measure of cellular water-insoluble mass-fraction that includes components of cell wall, cytoskeleton, denaturated proteins and other heat un-destructible polymers.

| | | |
| --- | --- | --- |
| 1. | Pre-weigh tubes (12 mL) | $\omega_1$ |
| 2. | Harvest culture sample | $V_s$ = 5 or 10 mL |
| 3. | Immediately boil it in water bath | 100°C for 5 min |
| 4. | Rigorously vortex once per min during boiling | vortex/min |
| 5. | Cool it down on ice/water bath | 0°C |
| 6. | Centrifuge | $5\times10^3$ *g* for 5 min at 0°C |
| 7. | Discard supernatant | |
| 8. | Dry the remains | overnight at 105°C |
| 9. | Weigh tubes | $\omega_2$ |
| 10. | Calculate the weight of the residuals | $(\omega_2 - \omega_1)$* |

* – should be corrected for weight loss by dry tube itself. Plastic tubes usually lose some weight unlike glass ones.

- *Measure of dry biomass (classical method)*

This is a measure of cellular biomass which presumably includes components of all cellular compartments together.

| | | |
| --- | --- | --- |
| 1. | Weigh filter (0.4µm) | $\omega_1$ |
| 2. | Harvest culture sample | $V_s$ = 10 mL |
| 3. | Immediately cool it down on ice/water bath | 0°C |
| 4. | Filter it out under vacuum | |
| 5. | Wash the filtrate with 3 volumes of ice-cold 0.9% NaCl | 30 mL of 0°C |
| 6. | Dry the pellet | overnight at 105°C |
| 7. | Weigh filter | $\omega_2$ |
| 8. | Calculate the weight of the biomass | $(\omega_2 - \omega_1)$* |

* – should be corrected for weight loss by dry filter itself.



SOP compiler: Dr. Maksim Zakhartsev, Institute of Biochemical Engineering (IBVT), University of Stuttgart, 70569 Stuttgart, Germany. T: +49 711 685 64 547, maksim.zakhartsev@ibvt.uni-stuttgart.de

- *Measure of dry biomass 'fixed' with cold MeOH [with 10% of the final concentration]*

This is a measure of cellular biomass which presumably includes components of all cellular compartments together. Presence of low concentrations of cold MeOH assists faster cooling of the sample together with partial metabolic denaturation (*i.e.* quenching), which assists preserving intracellular content from fast consumption due to residual metabolic activity. Also it is worth to note that low MeOH concentration does not result in salts precipitation.

| | |
|---|---|
| 1. Pre-weigh tubes (12 mL) | $\omega_1$ |
| 2. Fill it with MeOH and pre-cool | $V_{MeOH}$, -20°C |
| 3. Inject culture sample into tube with cold MeOH | $V_s$<br>$V_s$:$V_{MeOH}$ = 9:1=10% |
| 4. Immediately cool the sample on ice/water bath | 0°C |
| 5. Centrifuge | $5\times10^3$ *g* for 5 min at 0°C |
| 6. Discard supernatant | |
| 7. Re-suspend the pellet in 3 volumes of ice-cold 0.9% NaCl | 0°C |
| 8. Centrifuge | $5\times10^3$ *g* for 5 min at 0°C |
| 9. Discard supernatant | |
| 10. Dry the pellet | overnight at 105°C |
| 11. Weigh tubes | $\omega_2$ |
| 12. Calculate the weight of the residuals | $(\omega_2 - \omega_1)$* |

* – should be corrected for weight loss by dry tube itself. Plastic tubes usually lose some weight unlike glass ones.