SOP - Extraction of non-volatile, small, polar metabolites

The entire 400-ml cultures were centrifuged (17,700 × *g* for 15 min at 4°C). Cell pellets were immediately washed with 100 ml NaCl (0.9 % [wt/vol], anoxic for anaerobic cultures) and centrifuged (17,700 × *g* for 15 min at 4°C). The resultant pellets were immediately resuspended in 2 ml MeOH containing 0.02−0.05 mM ribitol, transferred in portions á 1 ml into Precellys® tubes (see above), shock frozen in liquid N_2 and stored at −80°C until further analysis.
Samples were thawed on ice before homogenization at −10°C with 3 cycles at a speed of 6,800 rpm for 30 s with breaks of 30 s, using Precellys24 homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France). Afterwards, 400 µl water were added to the sample, followed by a mixing step of 2 min at 2,000 rpm (MixMate; Eppendorf, Hamburg, Germany). Then, 250 µl chloroform were added and the sample mixed again for 2 min at 2,000 rpm prior to centrifugation (10,000 rpm for 10 min at 4°C). Subsequently, the polar phase was then transferred to 2-ml reaction tubes and dried at 14°C under vacuum. Samples were stored at −20°C until derivatization for GC-EI-MS analysis. For every growth condition 4 biological samples with 2 technical replicates each were analyzed.