

A method for metabolomic sampling of suspended animal cells using fast filtration

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Introduction

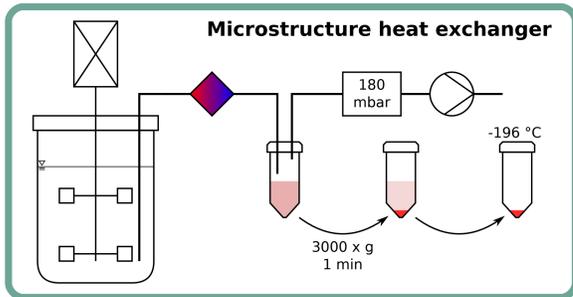
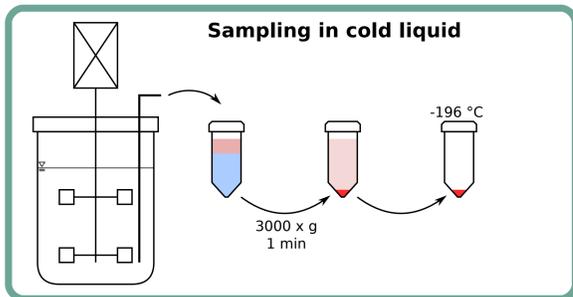
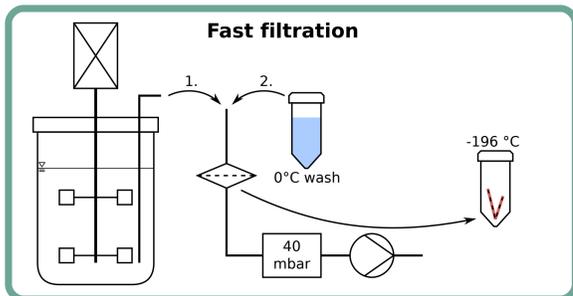
For intracellular metabolomic analyses it is of utmost importance to rapidly stop the organism's metabolism during sampling. This is to avoid false data due to residual enzymatic activity during sampling. Unlike for bacteria and yeast, there is not one generally approved protocol for metabolome sampling of suspended animal cells. A number of sampling procedures have been developed and described in literature but have not been compared in depth.

Here we describe a sophisticated metabolomic sampling method for suspended animal cells using a fast filtration protocol. The main requirements for the fast filtration method were to reduce the time for quenching and cell-medium separation while reducing cell disruption to a minimum. Additionally, the fast filtration method is compared to other sampling methods described in the literature.

Methods

CHO DP12 cells were cultured in shaker flasks (Corning Inc.) and 2 L Bioreactors (Biostat MD, Sartorius) to generate sample cell suspensions for the experiments.

The different quenching methods were used according to the literature. Methods tested include sampling with fast filtration^[1], sampling in cold saline solution^[2], sampling in cold methanol/AMBIC^[3] and sampling with a microstructure heat exchanger^[4].



For ¹³C-labeling experiments the cells were transferred to saline solution prior to sampling. ¹³C-labeled glucose was added to the cells followed by immediate sampling and the ratio of labeled and unlabeled glycolysis metabolites was determined.

LDH and ATP release from the cells was measured using plate tests. The intracellular energy metabolism was investigated using HILIC columns on a LC-MS system.

Results

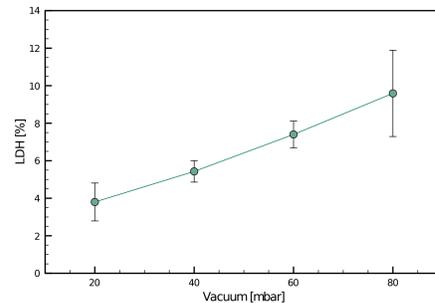


Figure 1 Determination of cell damage for fast filtration with different vacuums. Cell damage for reference sampling (centrifugation without cooling) was determined to be 2.56 ± 0.66 %. Cell damage increased with vacuum as expected

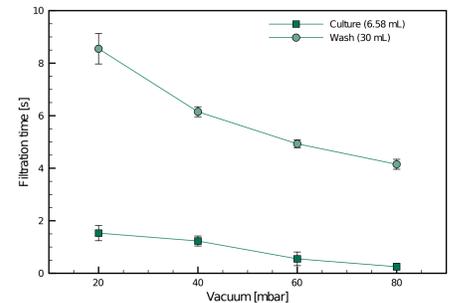


Figure 2 Time for fast filtration at different vacuums. The filtration time decreased with increasing vacuum. At 40 mbar the overall sampling time was less than 20 s

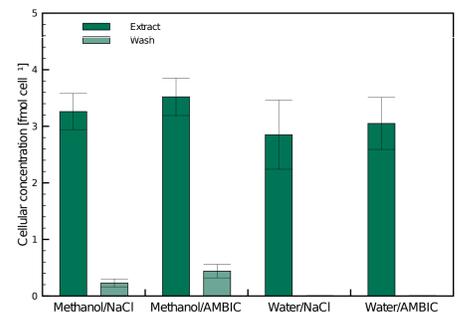
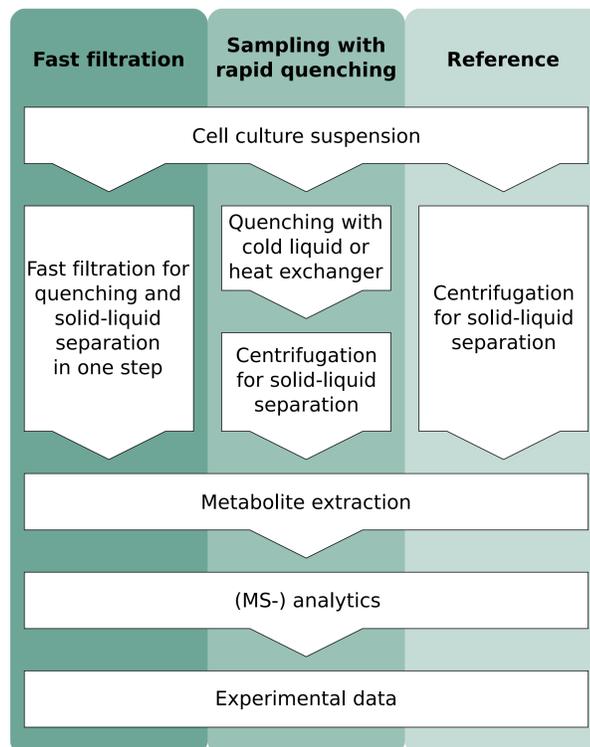


Figure 3 Metabolite release from cells during sampling. Methanol containing liquids provoked ATP release during sampling indicating exceeded cell damage

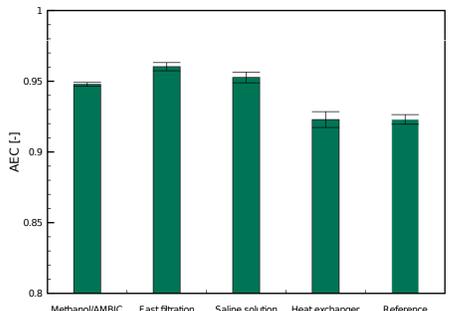


Figure 4 Adenylate energy charge as a measure of quenching efficiency. Sampling in cold liquid and fast filtration resulted in higher AEC values than sampling with the heat exchanger and reference sampling. Thus, indicating higher quenching efficiencies

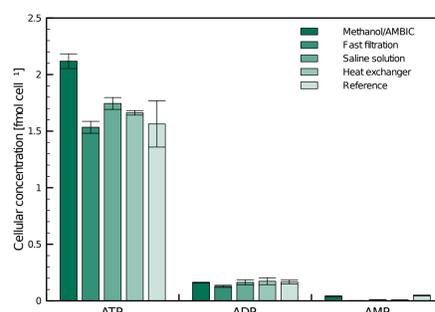


Figure 5 Metabolite concentrations in extracts after sampling with different methods. Overall concentrations were comparable. Only the ATP concentration when sampling with methanol/AMBIC differed slightly

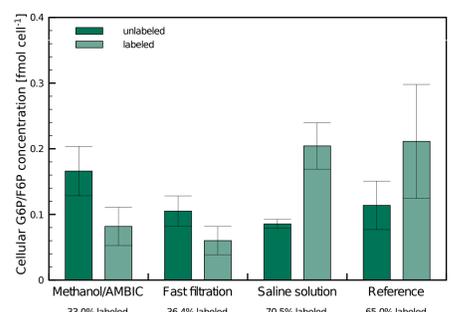


Figure 6 Quenching efficiency as determined using ¹³C-labeling experiments. Sampling with methanol/AMBIC and fast filtration exhibit comparably high quenching efficiencies. Saline solution and reference sampling are less efficient in terms of stopping the metabolism

Conclusions

Fast filtration for metabolome sampling

- Fast filtration is applicable for sampling of suspended animal cells.
- A vacuum of 40 mbar offers a good compromise of sampling time and low cell disruption.
- No residual medium components were found in extracts from cells on filters (data not shown).
- Extraction efficiency of cells on filters is comparable to extraction of cell pellets.

Comparison of sampling methods

- Concentrations of adenylates are comparable in extracts from all methods tested.
- AEC and ¹³C-labeling analysis show best quenching efficiency for fast filtration and methanol sampling.
- Metabolite bleeding from cells during sampling was observed when using methanol.
- In conclusion fast filtration is best suited for reliable metabolome sampling of suspended animal cells.