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Research Article

Automated fast filtration and on-filter quenching improve the intracellular metabolite analysis of microorganisms

To reliably determine intracellular metabolite concentrations in microorganisms, accurate sampling and sample inactivation strategies are crucial. Here, we present a method for automated fast filtration and on-filter quenching of microbial samples to overcome metabolite leakage induced by cold shock and significantly reduce the sampling and treatment time compared to manual filtration methods. The whole process of sampling, sample filtration, filter wash, and quenching of the filter with liquid nitrogen was finished in less than 6–15 s, depending on the experimental setup. By integration into an automated fast sampling device, we compared our method to the conventional methanol quenching method and showed that intracellular amino acid contents in *Escherichia coli* were significantly increased ($\geq$75%) with our fast filtration and on-filter quenching method. Furthermore, we investigated different filter types for the fast filtration and the efficiency of metabolite extraction from cells held on filters. Additionally, we found that the fast filtration behaves considerably different during exponential and nonexponential growth, probably due to variations of cell morphologies. Overall, we demonstrated that the automation of the fast filtration method significantly reduces the time for filtration and quenching and hence enlarge the number of metabolites that can be quantified with this leakage-free sampling method.

Keywords: *Escherichia coli* / Fast sampling / Metabolomics / Metabolite leakage / Methanol quenching

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1 Introduction

The analysis of intracellular metabolites in microorganisms has become an important tool of systems biology and metabolic engineering. Beside the attempts to understand more about cellular systems [1, 2], an increasing number of studies use metabolite analysis for the development of more efficient bioproduction strains and bioprocesses [3, 4].

For the analysis of intracellular metabolites, a number of steps have to be performed: fast sampling and subsequent quenching to stop the cells metabolism, separation of cells from their surrounding media, extraction of metabolites from the cells, and the analysis of the cell extracts [5, 6]. Herein, the sample quenching should be fast and effective to maintain the physiological state of the cells metabolism. This is generally done by spraying the sample into a cold solution, e.g. methanol-water [7], or by exposing the sample directly to an extraction solution [8]. The latter has the limitation that no separation of intra- and extracellular metabolites is possible. After the cold methanol quenching, a separation step is generally performed either by centrifugation [7, 9] or by filtration [10, 11].

Despite the efforts made to develop protocols for sampling, sample processing, and analysis of biological samples for metabolome analysis, some problems still remain unsolved. In this regard, the problem of metabolite leakage as a consequence of most quenching methods is still a critical issue. Several studies with different species of bacteria showed that exposing bacterial cells to a cold solvent solution caused severe leakage of intracellular metabolites into the quenching solution [9, 12–17]. For eukaryotes, this phenomenon was also shown for Chinese hamster ovary cells [18, 19] and for *Saccharomyces cerevisiae* [20]. One approach to resolve the problem of metabolite leakage is the application of a differential method [21]. First, a sample of the whole culture broth is quenched and intra- and extracellular

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Abbreviations: CDW, cell dry weight; **FFQ**, fast filtration and on-filter quenching

metabolites are pooled together. Subsequently, a cell-free sample is taken to determine the different metabolite pools by balancing. This method is able to deliver more reliable metabolomic data for metabolites that are absent or exist in a very low concentration in the medium. But, this method has strong limitations in the case of extracellular abundance due to the high excess of extracellular volume over the cells volume.

In the case of extracellular abundance of the target metabolites (e.g. in amino acid bioproduction processes), fast filtration was shown to be a powerful tool for an almost leakage-free intracellular metabolite analysis [12, 14, 19, 20, 22]. For this method, the sample is normally filtered manually on a vacuum filtration device, the filter is washed to remove residual medium and subsequently the filter is quenched by transferring it into liquid nitrogen or directly into an extraction solution. With this method, it is possible to separate the cells from the medium before quenching and thus avoid the leakage of metabolites into the extracellular medium. The drawback of this method is the prolonged sampling time needed for filtration of about 30 s. This limits the application of manual fast filtration methods to a certain set of metabolites, due to the fact that the turnover times of different metabolites can be <1 s for some glycolysis metabolites, although for many amino acids or tricarboxylic acid cycle metabolites it may last several minutes [1, 21, 23].

Fast sampling methods generally require a certain level of automation, but the automated fast sampling systems described in literature are all designed to perform sampling into cold or hot quenching solutions [24, 25]. However, the filtration was only done manually so far because there was no system developed to perform automated fast filtration. An automation of the filtration step can drastically reduce the critical time for the filtration process and thus enlarge the set of metabolites that can be quantified by this almost leakage-free sampling method. In the present work, we present a fast filtration method integrated into an automated sampling device to perform the sample filling, filtration and wash of the filter cake automatically, and to enable liquid nitrogen quenching of the filter in a semiautomatic manner. The used sampling system was able to perform both, fast filtration and cold methanol quenching simultaneously and thus a direct comparison of both methods was possible. The filtration method was tested in fermentation of *Escherichia coli* AN92 and analysis of intracellular amino acids. We investigated technical aspects of the fast filtration, i.e. the selection of suitable filters, but also the extraction of metabolites from filters and the influence of morphology changes in different culture conditions on the filtration process.

2 Materials and methods

2.1 Chemicals, strain, and medium

Chemicals were purchased from Sigma Aldrich (Germany), Roth (Germany), or Merck (Germany) and were of analytical grade. In this work, we used the recombinant *E. coli* strain AN92 (ME5355) ΔmetE (pQE80L), which has an introduced methionine auxotrophy. The strain was kindly provided by the group of Prof. Nediljko Budisa (Biocatalysis group, Berlin University of Technology). The strain was cultivated in minimal medium as described in Budisa et al. [26] with the following adaptations: the phosphate buffer was reduced to one-tenth of its original concentration, glucose was added to a final concentration of 24 g/L, and shikimic acid was supplied at 10 μM. The concentration of all amino acids was 0.2 g/L except for L-arginine (0.6 g/L) and L-methionine (0.04 g/L).

2.2 Cultivation

Precultures were grown in 100 mL shake flasks filled with 20 mL of the above described medium but with 4 g/L glucose. The cultures were incubated at 37°C and 160 rpm until an OD of one was reached. Bioreactor cultivations were carried out in a 3.7 L glass bioreactor (Bioengineering AG, Switzerland). Temperature (37°C), pH (7; titration with 5 M NaOH), and stirrer (700 rpm) were controlled automatically. The cultivation was carried out in batch mode with a working volume of 2.5 L and an inoculation volume of 5 mL from the preculture. The batch cultivation was limited by the L-methionine concentration, meaning the cell growth was stopped after consumption of methionine.

2.3 Sampling system and cold methanol quenching

An automated fast sampling system was used for fast sampling with cold methanol quenching and for the fast filtration method, respectively. For the methanol quenching, samples of 2 mL and simultaneously 8 mL of cold 60% v/v methanol solution (−45°C) were filled automatically into sampling tubes. No buffer was used in the quenching solution, as it was shown that this has no effect on the leakage behavior [15, 21]. The sampling time was less than 1 s and the sample temperature after quenching was maintained below −20°C as validated by temperature measurement. After quenching, the samples were centrifuged for 5 min (10 000 × *g*, −19°C, Heraeus Biofuge Stratos, Thermo Scientific, Germany), cell pellet and supernatant were separated and stored at −20°C for further processing.

2.4 Automated fast filtration and on-filter quenching (FFQ)

The fast filtration method was also implemented in the sampling system mentioned above. The custom-made filter module was made from stainless steel and is shown schematically in Fig. 1. The module was mounted between the sampling tube and the filling system (Fig. 2). A sample of approximately 2.1 mL was automatically drawn from the bioreactor and pressed through the filter by pressurized air (1.5 bar). The sample filtration was finished after 2 s and directly followed by two wash steps of the filter (2.1 mL each) of 5 s to rinse away residual medium components. After washing the filter, the sampling rack moved to the next position and the whole filter module was immediately filled with liquid nitrogen for filter quenching. The whole filtration process including two wash steps and the quenching of the filter was finished in less than 15 s. We used a combination of a prefilter (Pall glass fiber filter type A/E; 1 μm pore size; 25 mm diameter, Pall Corporation, USA) on top of a membrane filter (Pall Supor 800;

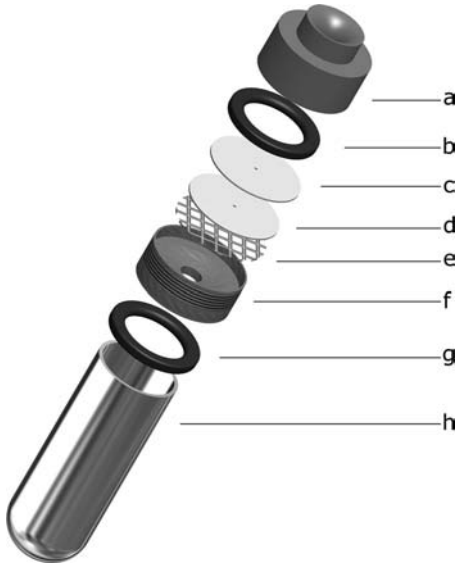

Figure 1. Filter module assembly. (a) Upper part of stainless steel housing. (b) O-ring seal. (c) Glass fiber filter. (d) Membrane filter. (e) Filter support mesh. (f) Lower part of filter housing. (g) O-ring seal. (h) Sampling tube.

0.8 μm pore size; 25 mm diameter, hydrophilic polyethersulfone) to achieve good separation within a short time. The wash solution contained 2 g/L 1,3-propanediol as internal standard and 10.2 g/L NaCl to adjust the ionic strength of the wash solution to that of the cultivation medium as proposed by Bolten et al. [14]. The frozen filter was then transferred into a centrifugation tube filled with 5 mL ethanol solution (75% v/v) at −20°C and stored at −20°C until metabolite extraction. The obtained filtrate was quantified gravimetrically and subsequently stored at −20°C until analysis.

2.5 Metabolite extraction and sample processing

Intracellular metabolites were extracted with the boiling ethanol method, based on protocols described by Gonzalez et al. [27] and Mashego et al. [28]. Cell pellets obtained by cold methanol quenching were extracted by adding 2 mL boiling 75% v/v ethanol followed by rigorous vortexing and incubation at 90°C for 3 min. The extracts were cooled on ice for at least 3 min and then centrifuged 5 min at 10 000 × *g* and −19°C. The supernatant was collected and the remaining cell pellet was subjected to the same extraction procedure a second time. The obtained supernatants were filtrated (PVDF, 0.22 μm pore size, Roth, Germany) and subsequently concentrated by solvent evaporation for about 5 h at 25°C (RVC 2–25 CD, Martin Christ Gefriertrocknungsanlagen GmbH, Germany). The obtained extracts from cold methanol quenching were corrected by the residual medium in the quenched pellets. Metabolite extraction of the filters obtained from fast filtration was done by heating the filter in the 5 mL extraction solution until boiling and then following the same protocol as described above. All dried extracts were resuspended in 500 μL deionized water and stored at −20 °C until analysis.

2.6 Analytics

Cell concentration was determined by gravimetric measurement of cell dry weight (CDW) and by measuring the OD at 600 nm. The correlation between CDW and OD was found to be CDW = 0.514 OD × g/L. Organic acids, alcohols and glucose were measured by HPLC (Kontron, Germany) with an Aminex HPX-87H column (300 × 7.8 mm) at 60°C with UV and refractive index detection. The mobile phase was H_2SO_4 (0.005 M). Amino acids were analyzed by HPLC using derivatization with the AccQ-Tag method (Waters, USA) and fluorescence detection as described in the manufacturer's manual. Measurement was done on an Ultimate-3000 HPLC system (Dionex, Thermo Fisher, Germany) with a binary gradient where eluent A was 140 mM sodium acetate with 0.1% v/v ACN and eluent B was 60% v/v ACN. The gradient was as follows: at 0 min 0% B; at 0.5 min 1% B; at 2 min 5% B; at 21 min 12% B; at 26 min 17% B; at 55 min 33% B; and remained from 62 min till 80 min at 100% B. All gradient changes were linear between the points given above. Separation was done on a Kinetex RP column (2.6 μm, C18, 100 × 4.6 mm, Phenomenex, USA) at 45°C, injection volume

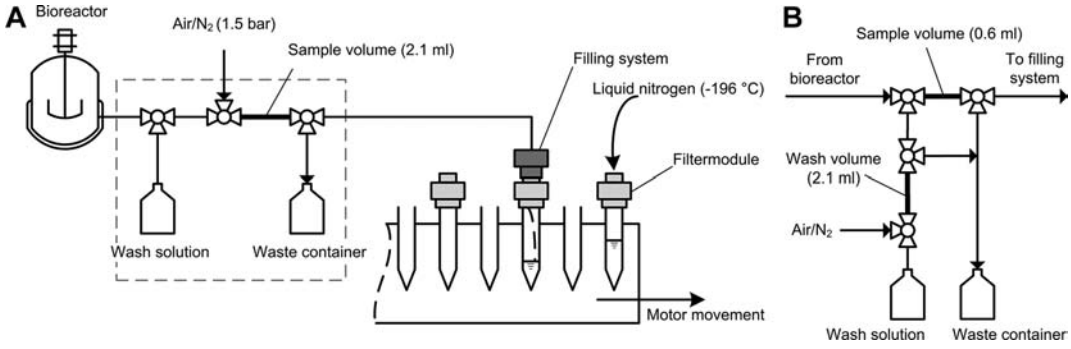

Figure 2. (A) Schematic view of the automated fast filtration method. Steps of the filtration process: (1) Dosing of the sample volume by filling the tube system from bioreactor to the waste container, either by overpressure in the bioreactor or by vacuum in the waste container. (2) Valves switching; filtration of sample by air pressure. (3) Dosing of wash solution as mentioned in step 1. (4) Filtration of wash solution. (5) Repetition of steps 3 and 4. (6) Motor movement and liquid nitrogen quenching. (B) Modified setup of the dotted box in Fig. 2A as described in Section 3.5.

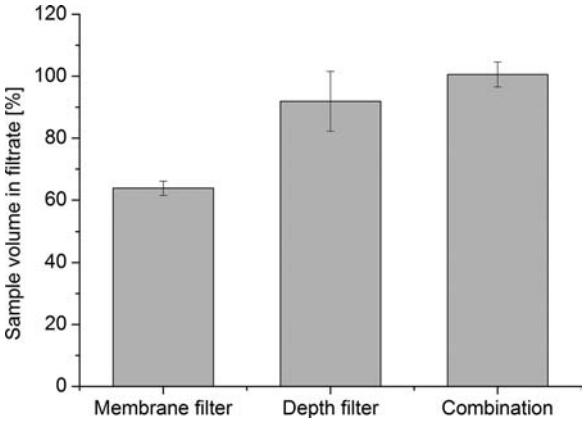

Figure 3. Comparison of filtrate volumes in the automated fast filtration with membrane filter (Pall Supor 800), depth filter (Pall glass fiber filter type A/E), and combination of both. The concentration of *Escherichia coli* cells was 2.24 g/L (cell dry mass) at exponential growth. Mean values and standard deviations were calculated from three technical replicates.

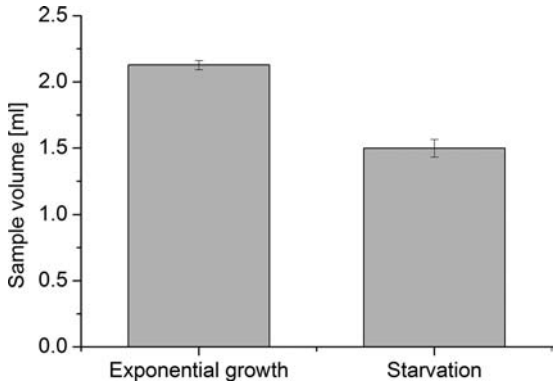

Figure 4. Filterable sample volume in the fast filtration within the given filtration time of 15 s with *Escherichia coli* in different growth phases. Biomass concentration was 1.99 g/L (exponential growth) and 2.31 g/L (methionine starvation). During methionine starvation phase, filter clogging was observed that led to a reduction of the filterable sample volume. This can be attributed to morphology changes in the culture resulting from the starvation. Mean values and standard deviations were calculated from two technical replicates, each with two analytical replicates.

was 10 μL. Derivatization efficiency was corrected using alpha-aminobutyrate as internal standard. Cysteine, glutamine, and tryptophan could not be detected; asparagine and serine were not separated and thus treated as one compound. The statistical analysis of data was done using Student's *t*-test.

3 Results and discussion

3.1 Comparison of depth filtration and membrane filtration for automated fast filtration

For the development of an automated fast filtration, the selection of a suitable filter type is crucial in terms of particle retention, loading capacity, and filtration time. Depth filtration as done by glass fiber filters is known to have high loading capacity and enables much higher flow rates than membrane filtration. On the downside, depth filters have a certain break through point, where additional particles are no longer retained by the filter and thus no complete separation of particles and filtrate is possible. Membrane filters can achieve complete retention of particles, but have significantly lower flow rates and may suffer from filter clogging due to the development of a filter cake during filtration.

In this study, membrane filters of 0.8 μm pore size as well as glass fiber filters (1 μm) were tested. The above-mentioned characteristics of the different filtration principles became clearly visible in our experiments. Figure 3 shows the percentage of sample volume in the filtrate. For equal cell concentration, the filtrate flux through the membrane filter was stopped at 63.8 $\pm$ 2.3% due to filter clogging. No severe filter clogging was observed with the depth filtration (91.9 $\pm$ 9.7%) and especially with the combination of glass fiber filter on top of the membrane filter (100.6 $\pm$ 4%), respectively. However, the former one showed no biomass retention at all, as the cells were washed out from the filter completely within the wash steps. With the combination of depth filter and membrane filter, complete particle retention was obtained and no filter clogging was observed. The glass fiber filter acts as a prefilter here, to reduce particle loading on the membrane filter and thus filter clogging. In contrast to manual fast filtration approaches, our automated fast filtration required increased filtrate flux due to a shorter filtration time, what was not achieved by the tested membrane filter. Glass fiber filters, as used by Volmer et al. [19] for manual fast filtration of suspended animal cells turned out to become damaged in our automated fast filtration method, probably due to pressure bumps in the pressure-driven filtration. Thus, the combination of prefilter and membrane filter was used for all automated fast filtration experiments.

3.2 Influence of cell morphology changes during starvation phase on the filtration process

Most studies on fast filtration for metabolite analysis are limited to exponentially growing cells [12, 14, 19, 20]. However, metabolite analysis in fermentation stages other than the exponential phase may be of special interest, since in some cases the stationary phase is related to important process issues, e.g. production of target products. To investigate if the culture condition has an influence on the filtration process, fast filtration was done in different growth phases of *E. coli*. Thus, the automated fast filtration was done with exponentially growing cells and compared to the filtration of starving cells that did not show any growth. It turned out that cells which are suffering from starvation showed significant filter clogging as shown in Fig. 4, where the sample amount that could be filtrated within the given filtration time was reduced to 68% (1.5 $\pm$ 0.07 mL) of the original sample size. The slight increase in biomass concentration from 1.99 g/L (exponential growth) to 2.31 g/L (methionine starvation) had no significant effect on filter clogging here (not shown). Thus, the reduction of sample volume can be attributed most likely to the morphology changes in the culture, as it is known that starving cells build cell agglomerates and change their size and shape [29]. The influence of cell morphology on microfiltration

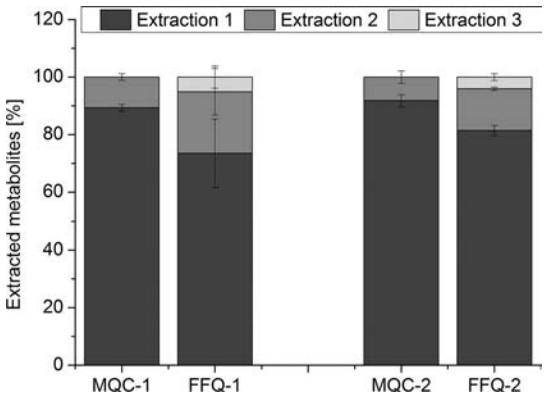

Figure 5. Extraction efficiency. Boiling ethanol extraction was performed with cell pellets from cold methanol quenching (MQC) and with filters from the fast filtration method (FFQ). For cold methanol quenching, two extractions were sufficient for a complete extraction, while for extraction of metabolites from filters a third extraction step had to be introduced. Metabolite extraction was performed in 75% v/v boiling ethanol solution for 3 min and subsequent cooling on ice. 1: Exponential growth; 2: Methionine starvation. Mean values and standard deviations were calculated from three technical replicates, each analyzed in duplicate (MQC) and from two technical replicates, each analyzed in duplicate (FFQ).

of microbial suspensions was discussed in detail by Foley [30]. Briefly, cell morphology can influence the filter cake compressibility, which in return directly affects the specific cake resistance and thus the filtrate flux, respectively. For a short filtration time, a high flow velocity is necessary and this was achieved by elevated pressure difference across the membrane. This resulted in a relatively high specific cake resistance as the resistance is strongly pressure dependent and rises with increasing pressure. The influences of morphology changes were clearly visible here, as these caused a considerably lower filtrate flux by the increased specific resistance. The problem of filter clogging during starvation phase can be overcome by the reduction of the original sample volume, as tested in later experiments. This underlines the need to adapt all sampling strategies and methods to the investigated organism and extends this necessity to the different growth phases of the culture. Especially for the fast filtration method, the cell morphology has a strong effect on the sampling process. Thus, for sampling during the stationary growth phase or phases with a certain nutrient limitation that are of major importance for many production processes, this issue has to be considered for transferring sampling methods developed for the exponential growth phase to other phases of batch or fed-batch processes.

3.3 Efficiency of intracellular metabolite extraction from filters

For the extraction of cell pellets from cold methanol quenching, 1–2 extraction steps are generally considered as a complete extraction [5, 9, 12–16, 19, 21, 31]. This could be also confirmed in our experiments (Fig. 5), where the extraction of cell pellets from cold methanol quenching was finished after two extraction steps. Here, 90.6 ± 1.7% of the metabolites were extracted in one step and after the second step, no further metabolites were extracted.

To extract intracellular metabolites from cells bound to a filter, two extraction steps were not sufficient, as there were only 95.4 ± 0.8% of metabolites recovered (Fig. 5). This could be corrected by applying a third extraction step on the filters. This lower extraction efficiency most likely originates from two facts: cells or metabolites bound to a filter material are less accessible for the extraction solution than suspended cells and second the solvent uptake by swelling of the filter materials reduces the recovery of the extraction supernatant after centrifugation. Since the metabolite extraction depends on numerous parameters, such as the extraction method used, or the filter types used for fast filtration, we recommend validating the efficiency of the applied extraction method for the respective experiment when extracting metabolites from filter-bound cells.

3.4 Comparison of the FFQ method and the method of cold methanol quenching with subsequent centrifugation

3.4.1 Exponential growth phase

Manual fast filtration was already applied by different groups with a vacuum filtration device [12, 14, 19]. It was shown that fast filtration is a suitable method for analysis of many intracellular metabolites (e.g. amino acids) and that this method can avoid the leakage of metabolites from the cells interior almost completely. To compare our automated fast filtration method with the known manual methods, intracellular amino acid concentrations were determined from samples taken with the cold methanol quenching method and from samples obtained by automated fast filtration with on-filter quenching. Both sampling methods were applied in parallel, so that intracellular concentrations can be compared between both methods. Figure 6 shows different intracellular concentrations of some typical amino acids obtained from both sampling and quenching methods during the exponential growth phase of *E. coli*. In Table 1, all measured intracellular amino acids are listed for both methods. The intracellular concentrations (content) of amino acids were found to be significantly higher with the FFQ method, which can be attributed to severe leakage of amino acids in the cold methanol quenching method. The average loss of intracellular amino acids with the cold methanol quenching method was found to be 75 ± 16% of the amount found with the FFQ method. These findings are in accordance with the findings of Bolten et al. who found 84% loss for *E. coli* K12 [14].

To prove that the higher concentrations obtained by the FFQ method did not originate from residual medium components, which have not been washed away from the filter, the concentration differences between data from the FFQ method and data from the cold methanol quenching method (i.e. the leaked metabolites) were compared with the extracellular amino acid concentrations. The amount of medium required to compensate this concentration difference was significantly different among the measured amino acids ($p \leq 0.001$). This denotes that the data are not affected by residual medium on the filter, which is

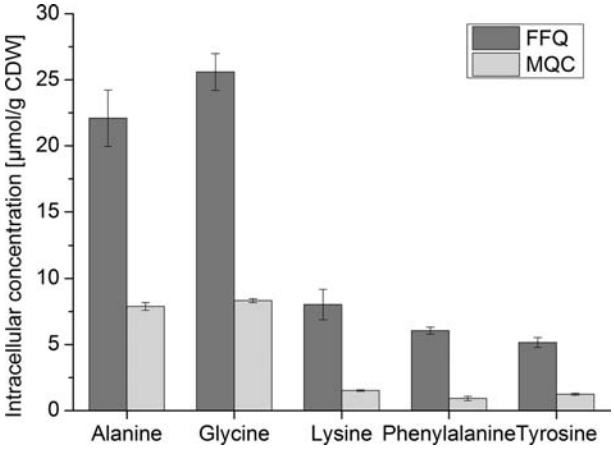

Figure 6. Intracellular amino acid content during exponential growth of *Escherichia coli* AN92 obtained by automated fast filtration with on-filter quenching (FFQ) and by cold methanol quenching followed by manual centrifugation (MQC). Means and standard deviations were calculated from two technical replicates, each with two analytical replicates (filtration) and from three technical replicates, each with two analytical replicates (methanol quenching). Excerpt data from Table 1.

further supported by the study of Kim et al. [20] as they showed that washing the filter with twice the sample volume removes all relevant medium.

3.4.2 Starvation phase

Samples obtained during methionine starvation showed similar results in terms of metabolite loss and intracellular concentrations as during exponential growth (Table 1). The average loss of metabolites with the cold methanol quenching method was slightly higher (82 ± 19%) than during exponential growth, which is within the error range. Due to the difficulties in the filtration of culture under starvation (see Section 3.2), a complete removal of the residual medium from the filter could not be ensured. Nonetheless, the similarity to the data from exponential growth phase indicates realistic intracellular concentrations here.

3.5 Reduction of sampling and treatment time by method optimization

To further reduce the total time for filtration and quenching, the FFQ method was optimized by a more effective setup (Fig. 2B). By adding a separate loop volume, the sample and wash solution could be dosed simultaneously and moreover, the volumes for sample and wash solution could be changed independently. In the new setup, the sample volume was reduced to approximately 0.6 mL while the wash volume remained at 2.1 mL. This resulted in an increased ratio (3.5) of wash solution to sample, which allowed combining both wash steps into one single step with even improved filter washing. After dosing the sample and the wash solution simultaneously, both were filtrated in one step, so that the time for the complete procedure including on-filter quenching needed no longer than 6 s. To avoid mixing of both fluids on their way to the filter module, an air gap of about 1 mL was introduced between the sample and the wash solution, and thus the back-mixing of the sample into the wash solution was negligible. As an added advantage, the problem of filter clogging during starvation phase (see Section 3.2) could be avoided completely by the decrease of the sample volume. It was shown that

Table 1. Intracellular amino acid concentrations (content) obtained by automated fast filtration with on-filter quenching and by cold methanol quenching followed by centrifugation for *Escherichia coli* AN92 cells during exponential growth and methionine starvation. Concentrations were calculated from amino acids in the cell extracts and the cell dry weight of the filtered sample, determined by internal standard analysis. LOD was 0.1 μmol (g cell dry weight)$^{-1}$. Means and SDs were calculated from two technical replicates, each with two analytical replicates (filtration) and from three technical replicates, each with two analytical replicates (methanol quenching).

	Exponential growth μmol (g cell dry weight)$^{-1}$		Starvation μmol (g cell dry weight)$^{-1}$	
Compound	Filtration	Methanol quenching	Filtration	Methanol quenching
Alanine	22.1 ± 2.1	8.6 ± 1.3	28.5 ± 3.3	10.0 ± 3.1
Arginine	17.8 ± 1.2	7.6 ± 1.0	21.2 ± 3.6	6.6 ± 1.3
Asparagine/serine	5.4 ± 0.5	<0.1	4.5 ± 0.4	3.4 ± 0.9
Aspartate	3.1 ± 0.1	1.9 ± 0.7	5.5 ± 0.6	< 0.1
Glutamate	145.9 ± 3.5	8.0 ± 2.1	312.1 ± 11.6	22.2 ± 4.8
Glycine	25.6 ± 1.4	8.3 ± 0.2	27.8 ± 1.0	7.7 ± 0.5
Histidine	7.8 ± 0.7	0.7 ± 0.2	9.0 ± 1.0	0.9 ± 0.3
Isoleucine	6.3 ± 1.7	2.3 ± 0.4	21.6 ± 5.2	4.2 ± 1.0
Leucine	6.6 ± 0.7	1.4 ± 0.1	7.6 ± 1.1	0.7 ± 0.3
Lysine	8.0 ± 1.1	1.6 ± 0.1	9.0 ± 1.6	0.6 ± 0.2
Methionine	<0.1	0.1 ± 0.0	<0.1	<0.1
Phenylalanine	6.0 ± 0.3	1.0 ± 0.2	6.6 ± 1.3	<0.1
Proline	13.6 ± 2.1	3.4 ± 0.6	18.1 ± 0.9	3.7 ± 1.2
Threonine	2.7 ± 0.4	0.4 ± 0.1	0.3 ± 0.3	<0.1
Tyrosine	5.2 ± 0.4	1.2 ± 0.1	5.5 ± 0.9	0.7 ± 0.5
Valine	6.1 ± 0.8	1.7 ± 0.2	4.6 ± 0.8	1.0 ± 0.3

with only a few modifications, the total time for the FFQ procedure was reduced from 15 to 6 s, what evidently underlines the high potential of automation for a fast and reliable metabolome sampling using the filtration method demonstrated in this work.

4 Concluding remarks

A method for automated fast filtration with on-filter quenching was developed to perform the whole sampling process for intracellular metabolite analysis. Sampling from the bioreactor, sample filtration, filter wash, and quenching of the filter with liquid nitrogen was completed within 6–15 s, depending on the experimental setup. The automation of the filtration and washing process and the direct on-filter quenching of the cells metabolism drastically reduced the total sampling time and thus allowed a more reliable determination of intracellular amino acids in *E. coli*. Furthermore, the automated fast filtration enables almost leakage-free intracellular metabolite analysis of many other metabolites with shorter turnover times, which was not possible with manual fast filtration methods. Technical aspects of the automated fast filtration were investigated, i.e. the selection of a suitable filter type, where we found a combination of a prefilter and a membrane filter is the best solution for fast pressure-driven filtration. We discussed possible pitfalls, e.g. the influence of cell morphology on the filtration process and the efficiency of metabolite extraction after fast filtration. We recommend these aspects to be carefully investigated and adjusted for the respective experiment. Another promising advantage of our newly developed method is the integration into an automated fast sampling system, which allows the parallel application of fast filtration and cold methanol quenching in the same experiment. This combination of different sampling and quenching strategies in one experiment offers new alternatives for the experimental design of future metabolomic studies.

Practical application

In this work, we present an automated fast filtration and on-filter quenching (FFQ) method for a more reliable intracellular metabolite analysis of microorganisms, especially to overcome the key problem of cold-induced leakage of metabolites in conventional methods. Technical aspects of the fast filtration, quenching, and metabolite extraction with the FFQ method are described and discussed. With the optimized system, the whole process of sampling from a bioreactor, filtration, washing, and quenching of cells can be finished within 6–15 s automatically, representing a drastic reduction of the sampling and treatment time of normally $\geq$30 s in conventional filtration methods. We demonstrated the significantly improved efficiency of the new method for the determination of intracellular amino acid content of *Escherichia coli* growing under different conditions. The method and equipment can have a wide range of practical applications in bioprocess monitoring and optimization, metabolic engineering of cells, and systems biology in general.

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The authors have declared no conflict of interest.

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