An automated home-built low-cost fermenter suitable for large-scale bacterial expression of proteins in *Escherichia coli*

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We have developed an automated fermentation system for cost-efficient upscaling of protein expression in bacteria. The system, built for use by nonbiotechnologists, can be assembled mostly from standard laboratory equipment and allows a largely unattended growth of bacteria to OD 25 (at 600 nm) in a 12 L vessel. The typical yield of 250–350 g of wet weight cell pellet per run, which is equivalent to the biomass obtained from 250 shake flask cultures containing 400 mL Luria-Broth medium each, facilitates the production of large amounts of purified recombinant protein without the laborious need for optimization of expression and purification conditions.

Many biochemical and biophysical studies of proteins depend on the availability of milligram quantities of highly purified proteins of interest. Heterologous expression of recombinant proteins in *Escherichia coli* is a practical and cost-effective tool for protein production, but standard shake flask cultures often yield insufficient amounts of the desired protein in its functional form. Therefore, tedious optimization procedures have been commonly applied, which include variation of the expression construct, the use of alternative bacterial expression strains, or change of culturing and lysis conditions (1–5). In principle, the simplest approach for increasing the on-hand amount of purified protein is to raise the feedstock; that is, scaling up bacterial culture volume and cell density. However, this problem-solving strategy usually requires expensive high-end fermentation systems and expertise to operate such equipment. Since neither is usually available in standard biology laboratories, this represents a common constraint. Here, we present the design and utilization of a semi-automated fermentation system that is mostly assembled from standard laboratory equipment. Thus, this system combines cost-efficiency with a minimum requirement for manual intervention.

The basic setup of the fermenter is shown in Figure 1. The 12 L fermentation vessel (Cat. no. 2600-0012; Nalgene, Rochester, NY, USA) is placed in a thermostated water bath (LAUDA, Instrumenten-Gesellschaft, Zurich, Switzerland). To allow for rigorous mixing during the fermentation run, an overhead stirrer (Model no. 741; Heidolph, Schwabach, Germany) is mounted that holds the stirring shaft with a propeller. Compressed air from local supply is filtered and pre-warmed by passing through a heat exchanger located inside the water bath. The humid exhaust air carrying a significant amount of bacteria is condensed in a cold trap for sterilization. During the fermentation run, the pH is constantly measured by a standard pH meter with analog output (Model no. 691; Metrohm, Zofingen, Switzerland) connected to a control unit. This control unit was purchased from Conrad Electronic (Wollerau, Switzerland) and was customized for our purpose (see Supplementary Material available online at www.BioTechniques.com). Similar control units may be available from other commercial suppliers, or may be self-assembled on a circuit board from the individual electronic parts. The control unit receives an input signal from the pH meter and switches the peristaltic pump (Model no. MC-MS/CA4; Ismatec, Glattbrugg, Switzerland) that titrates sodium hydroxide solution, thereby keeping the medium at neutral pH that otherwise would acidify during bacterial growth when using glucose as the major carbon source. In our first test runs with the fermentation system, we manually monitored cell growth, that is, by photometric determination of the optical densities of samples taken from the culture at different time points. We observed a linear correlation between cell density of the culture and total amount of titrated base that was automatically added by the control unit for pH stabilization (Figure 2A). Hence, the control unit was adapted for recording the number of pump turns causing base addition, which allowed for calculation of approximate values for actual cell densities in the vessel. Accordingly, the control unit was re-programmed and set up to also trigger the induction of bacterial expression at around OD_{600nm} 10 by turning on a pipet aid (PIPETBOY acu, Integra Biosciences, Wallisellen, Switzerland), thus enabling for automated addition of inducer solution to the growth medium once the desired cell density had been reached. Further, glycerol was added synchronously to sodium hydroxide solution (base) via a discrete tubing of the same pump, thereby providing an additional carbon source commensurate with bacterial growth. To prevent excessive foam production, a separate peristaltic pump was activated for a few seconds by the control unit in regular intervals of 20 min. A typical recording of the fermentation process is shown in Figure 2B. The OD_{600nm} correlated well with the amount of added base until after addition of the inducer, where the need for titration with sodium hydroxide ceased while the cells continued to divide.

Using the automated fermentation system, we expressed various isoenzyme combinations and mutants of AMP-activated protein kinase (AMPK) (6), brain-type creatine kinase (B-CK) (7), and His-TEV protease (8). We reproducibly obtained a biomass of

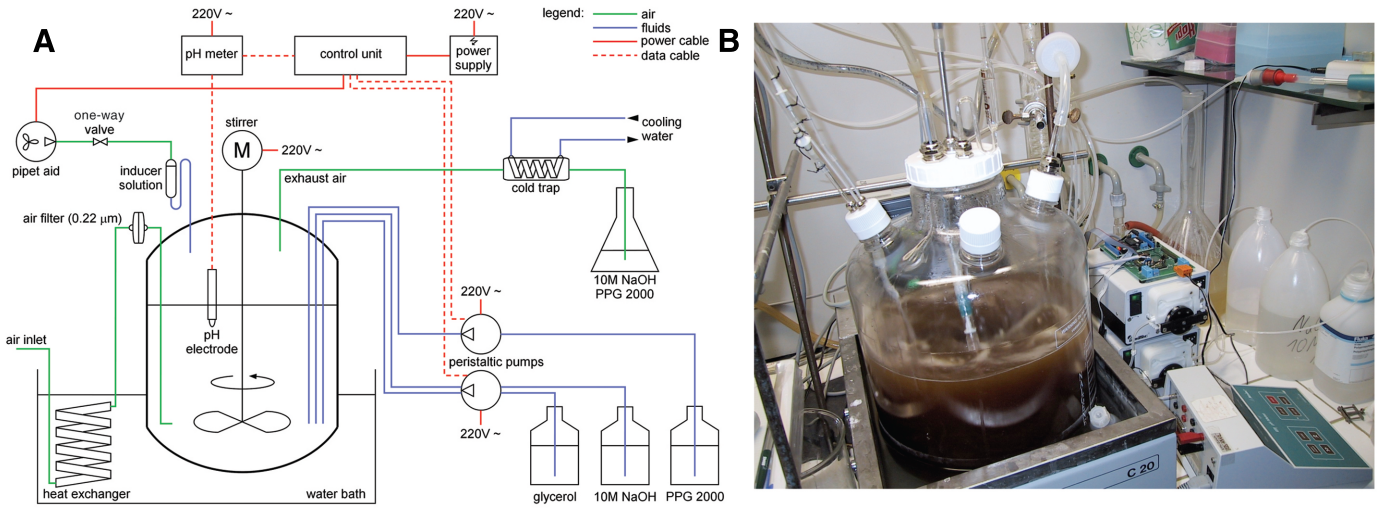

Figure 1. The fermenter setup. (A) Schematic overview. The fermenter consists mainly of three subsystems: First, the culture vessel including water bath, air inlet/outlet, and stirrer. Second, a pipet aid retaining the inducer solution and two peristaltic pumps with tubings connected to glycerol, base, and antifoam solution. Third, a pH meter and a control unit to monitor and adjust the culture conditions via the pH electrode and the pumps/pipet aid. A more detailed description of the parts and interplay is given in the text and the Supplementary Material available online at www.BioTechniques.com. (B) Photograph of the setup in a laboratory hood. For further details of the design please see Supplementary Material.

250–350 g wet weight per run with 10 L starting volume of growth medium (Supplementary Table S1). On average, the amount of purified enzyme per liter of medium [after first column, either using Blue Sepharose (GE Healthcare, Otelfingen, Switzerland) or immobilized-metal affinity chromatography] was increased well above 10-fold compared with the reported values derived from shake flask cultures (6–8). This gain constitutes a significant advantage over shake flask culturing as clarified by the following example calculation. If intending to grow 10 L bacterial culture in 2 L shake flasks, this would require 25 flasks containing 400 mL medium each (to maintain the recommended 1:5 ratio of medium and air). However, the resulting biomass of a single fermentation run is 10× higher per liter and would therefore correspond to the yield from 250 flasks. The explanation for such boosted overall yield of biomass is very simple, as 10-fold higher cell densities were easily reached in the efficiently aerated and pH-controlled environment of the fermentation vessel. Thus, the increased total cell mass entirely accounts for the increased overall amount of purified protein-of-interest without significantly altering protein yield per cell mass.

Depending on the application and the desired amount of purified protein, the whole cell pellet obtained from a single fermentation run may be used all at once. However, we (and many other laboratories) rather have a constant need for a certain freshly purified protein that is not conveniently obtained in sufficient yield from small-scale shake flask culturing. Hence, we prefer to divide the large cell pellet from a single fermentation run into smaller aliquots of 15–30 g each that are stored frozen until usage. This corresponds to the cell mass obtained from 11–30 shake flasks, given the typical yield of 1–1.4 g wet weight per 400 mL shake flask culture (see calculations in the previous paragraph). Compared to repetitive single small-scale expressions that may accumulate to a similar cell mass, the process of apportioning a larger pellet from a single fermentation run obviously offers significant time and work savings. More importantly, batch-to-batch variability of the subsequent protein purification results, which is a problem that is commonly observed after shake flask culturing, can be eliminated. Thus, optimization of protein purification strategies is much less affected by fluctuations of the starting material.

The automated fermentation system, as described herein, offers attractive upscaling capabilities for bacterial expressions in developing countries and laboratories lacking expertise in biotechnology. Assuming that most components of the fermentation system are usually present in average molecular biology laboratories, the acquisition costs are less than U.S.$2250 (Supplementary Table S2), which compares very favorably to professional systems (costing more than U.S.$10,000). However, it should be noted that a workshop collaboration might be necessary for the initial assembly of parts. Costs may be further reduced to a few hundred dollars if using second-hand laboratory equipment or alternative material, such as a drilling machine instead of the professional stirrer, or a cheap aquarist air pump instead of the pipet aid. Additionally, if fermentation capacity is not needed, most parts of our system can be used elsewhere in the laboratory for their specific purposes, thereby saving laboratory space, another common constraint. We have also used the commercial 5 L fermentation system Minifors (Infors AG, Bottmingen, Switzerland) that is worth U.S.$20,000 and capable of producing 200–250 g wet weight cell pellet using similar expression conditions. This professional system did not offer any significant advantages in comparison to our self-assembled fermenter, while featuring a lesser degree of automation. Therefore, smaller laboratories and those with more limited budgets could take advantage of the fermentation

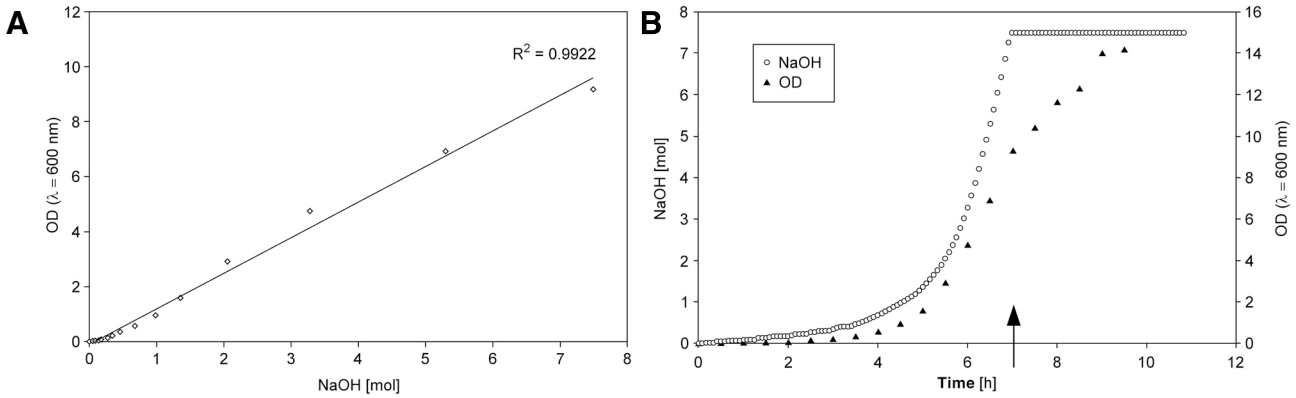

Figure 2. Base titration and growth of the bacterial culture. For stabilization of the pH at 7.1, base (10 M sodium hydroxide solution, NaOH) was added automatically during fermentation. Samples of the culture were taken manually at regular intervals for determination of the optical density using a photometer set at 600 nm wavelength (OD_{600nm}). (A) Correlation between OD_{600nm} and the amount of titrated base during fermentation before induction. (B) Graph showing OD_{600nm} of the bacterial culture versus amount of titrated NaOH and OD_{600nm} during a typical fermentation run. The arrow indicates the time point of induction of protein expression by IPTG.

system described herein for growth of bacterial cultures to overcome the bottleneck of time-consuming optimization. Recently, an alternative low-cost fermenter has been described (9). This latter system, however, still requires manual intervention for pH control, determination of optical densities, and induction, whereas our fermentation system basically works in an unattended manner. Such automation enables the researcher to express the desired protein overnight and use some parts of the fermenter (peristaltic pumps, pH meter, pipet aid) for protein purification or other applications during the day.

In conclusion, our system provides a low-budget solution for production of several hundred grams of bacterial cell mass in a semi-automated setup within one day.

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COMPETING INTERESTS STATEMENT

The authors declare no competing interests.

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