E.coli scale-up fermentation from mini to laboratory bioreactor

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Abstract

Bioprocess scale-up consists in transfer a new or improved process from the laboratory to production scale. Using a small-scale bioreactor is the first step to develop or improve a bioprocess, which decreases costs while mimicking the large scale conditions. In the current work two options of small-scale bioreactors with different scales have been used to study the scalability between them. A typical *E. coli* fermentation was performed in Applikon's bioreactors with nominal volumes of 3 L and 500 mL. The conditions of the cultivation were the same for both scales. The results were evaluated based on optical density (OD_{600}) measurements and process control. In this study, parameter control and cell growth profile exhibited the same behavior in both scales.

Introduction

he commercial production of many biotechnology products like antibiotics, alcohols, amino acids, enzymes and organic acids, can only be achieved when the process is optimized to a final bioreactor scale on the order of hundreds of thousand liters [1]. First, the experiment is developed in a small-scale to generate a solid understanding of the process and just then taken to a larger scale [2].
During the scaling-up process it is critical to maintain the same physical and chemical conditions. Chemical conditions include among others the pH, dissolved oxygen level, concentration of medium components and concentration of toxic metabolites. The physical conditions are related to the bioreactor configuration and the power supplied [3].
A wide range of small-scale fermenters can be used for bioprocess optimization. One of the strategies used for scaling up between stirred tank bioreactors is based on similar geometric configuration according to the following equation: $D_{T2}/D_{T1} = (V_{L2}/V_{L1})^{1/3}$ (1), where D_T is the vessel internal diameter; V_L is the working volume; 1 and 2 refers to the bioreactors. Similar geometric configuration facilitates the duplication of the mixing patterns, which is a critical parameter during scaling-up [3].
In the present work two options of small-scale fermenters were tested for scalability. The same gassing strategies were applied with spargers, impellers and a constant vessel ratios. For this end, a typical *E. coli* fermentation was performed using a 3 L laboratory bioreactor with a working volume of 2 L and a 500 mL bioreactor with a working volume of 400 mL.
The 3 L laboratory bioreactor simulates a stirrer aeration, mixing patterns and parameters closer to the ones found on a large-scale. On the other hand, the 500 mL bioreactor uses less bench space, less material and since it is smaller it can be autoclaved faster and a smaller autoclave could be used [4].
The aim of this work was to investigate the scalability between a 500 mL and a 3 L bioreactor, evaluating the online process parameters and the growth profile achieved during the cultivation in these two small-scale options.

Materials and methods

E. coli batch fermentations were performed in a 500 mL and in a 3 L bioreactor. The same configuration was used for both bioreactors with the same initial pre-culture and culture media. Furthermore, the same set points were used for the parameters that were controlled namely pH, dissolved oxygen (dO_2) and temperature. The biomass growth was compared by measuring the optical density at 600 nm (OD_{600}) at several time points during the fermentation.

A | Geometric similarity
To confirm the similar geometry between the two vessels the equation (1), above described was used:

1 - 3 L vessel 2 - 500 mL vessel	D_{T1} – 130 mm D_{T2} – 71 mm	V_{L1} - 2000 mL V_{L2} - 400 mL	$D_{T2} / D_{T1} = (V_{L2} / V_{L1})^{1/3}$

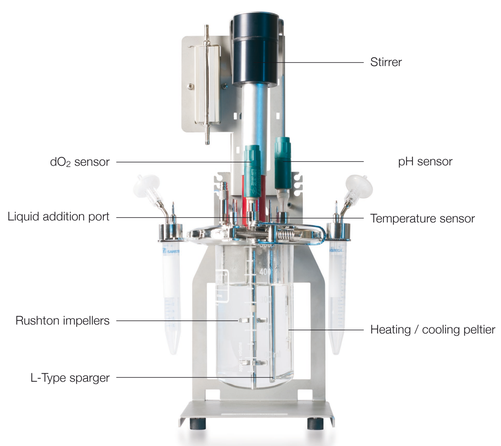
B | Microorganism and Media
- A pre-culture of *E. coli* K12 free from plasmid was grown in LB medium from a glycerol stock. The pre-culture was grown in a resonance acoustic incubator (RamBio, Applikon Biotechnology) for 16 h, at 30 ºC.
- Medium LB 2x was used for *E. coli* fermentation (20 g/L tryptone; 10 g/L yeast extract; 20 g/L NaCl. After dissolution of all the media components, pH was adjusted to 7 with NaOH 0.1 M solution).
- Bioreactors were inoculated using the same pre-culture with an initial OD_{600} of 0.2.

C | 500 mL MiniBio
- A 500 mL glass autoclavable bioreactor from Applikon Biotechnology was used for *E. coli* during batch fermentation with a working volume of 400 mL. The bioreactor was controlled using a my-control (Applikon Biotechnology) with dedicated software for process control. The assembly of the bioreactor is depicted in Figure 1A. The vessel was equipped with 2 rushton impellers, a L-type sparger with an open pipe, dO_2, pH and temperature sensors, sampling septum and a liquid addition port for pH control.
- Samples were taken by withdrawing 0.2 mL of culture through the sampling septum with a sterile syringe. The OD was measured with a spectrophotometer (Bischoff LAMBDA 1000) at 600nm.

D | 3 L Bioreactor
- Applikon's 3 L glass autoclavable bioreactor was used for *E. coli* batch fermentation with a working volume of 2 L. The general set-up of this bioreactor is depicted in Figure 1B and it is the standard set-up for a typical microbial cultivation. The inserts used were the same as in the 500 mL bioreactor. The bioreactor was built with dO_2, temperature and pH sensors and these parameters were controlled with an ez-control (Applikon Biotechnology). Aeration was performed with a L-type sparger with an open pipe, the vessel was assembled with 2 rushton impellers, sampling septum and addition port for pH control.
- Samples were taken by withdrawing 1 mL of culture through the sampling septum with a sterile syringe. The OD was measured with a spectrophotometer (Bischoff LAMBDA 1000) at 600nm.

E | Actuators and set points
- The dO_2 was controlled upwards using an air valve at the set point of 30% air saturation for both bioreactors.
- The pH set point of 7.0 was controlled upwards with ammonia liquid solution (25 % V/V).
- The set point for temperature was 37 ºC. The temperature of the 3 L bioreactor was controlled with a heating jacket and a cold finger while for the 500 mL, the temperature was controlled with a heating/cooling peltier.
- Stirrer speed was set to 1000 rpm and 2000 rpm for the 3 L and 500 mL, respectively. The KLa measured for the conditions described is ca. 217 h-1 for both bioreactors [4].
- The process values of the controlled parameters logged from both controllers (ez-control - 3 L bioreactor; my-control - 500 mL bioreactor) were acquired with SCADA software from Applikon Biotechnology.

1A **400 mL working volume**



1B **2000 mL working volume**

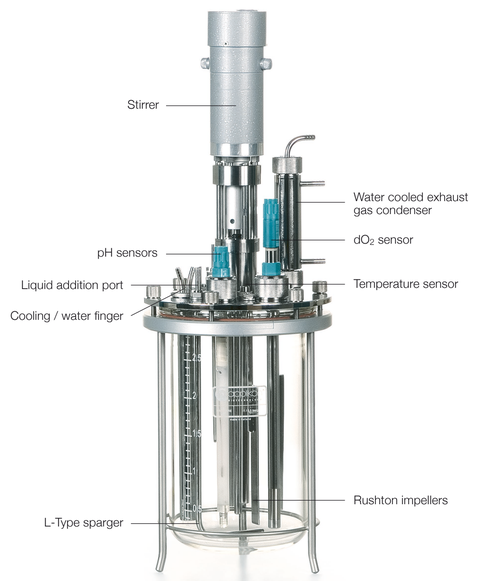


Figure 1 | A | Set-up of mini-Bio depicting gas inlets heating/cooling peltier and sensors to control temperature, pH and dissolved oxygen. B | Stirred bioreactor 3 L with pH, temperature and dissolved oxygen measurement sensors.

References

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[2] Junker BH, "Scale-Up Methodologies for Escherichia coli and Yeast Fermentation Processe", J. Bio. Bioeng. 97(6) 2004: 347–364.
[3] B. H. Junker, "Scale-up methodologies for Escherichia coli and yeast fermentation processes", J. Biosci. Bioeng., vol. 97, no. 6, pp. 347–364, 2004.
[4] ApplikonBiotechnology, 2013.
[5] Lee, S. J., Lee, D., Kim, T., Kim, B., Lee, S.Y., "Metabolic Engineering of Escherichia coli for Enhanced Production of Succinic Acid, Based on Genome Comparison and In SilicGene Knockout Simulation", A. Soc. for Micro., Dec. 2005, pp. 7880–7887.

Results and discussion

To investigate the scalability between a 3 L and 500 mL bioreactors, parallel *E. coli* batch fermentations were performed for 7 h. The results from the optical density measured at different time points are presented in Figure 2.

2

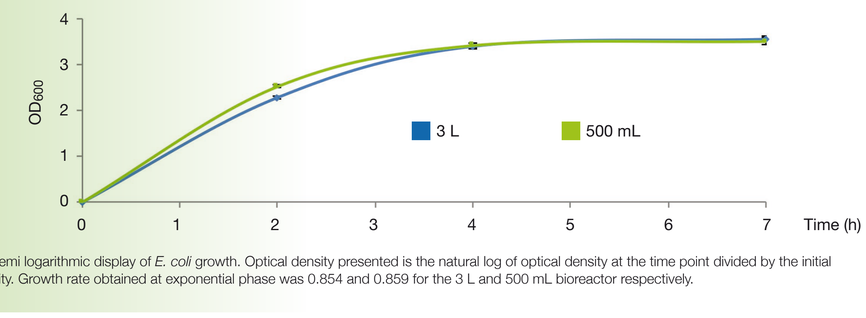

Figure 2 | Semi logarithmic display of *E. coli* growth. Optical density presented is the natural log of optical density at the time point divided by the initial optical density. Growth rate obtained at exponential phase was 0.854 and 0.859 for the 3 L and 500 mL bioreactor respectively.

The growth curves obtained after 7 h of batch fermentation are very similar for both scales. The final biomass achieved for the 3 L and 500 mL fermenters was 7.12 ± 0.07 and 6.81 ± 0.09, respectively.
The results from this work show that the growth conditions inside a 3 L and 500 mL bioreactors are close and that the results from both scales are reproducible between them. It was shown that the same K_La value can be obtained for both scales and that both vessel share similar geometry making them efficient options for scaling-up/down.

Controlled Parameters
The comparison between the parameter control using the ez-control (3 L bioreactor) and the my-control (500 mL bioreactor) is depicted in Figures 3A, 3B and 3C.

3A

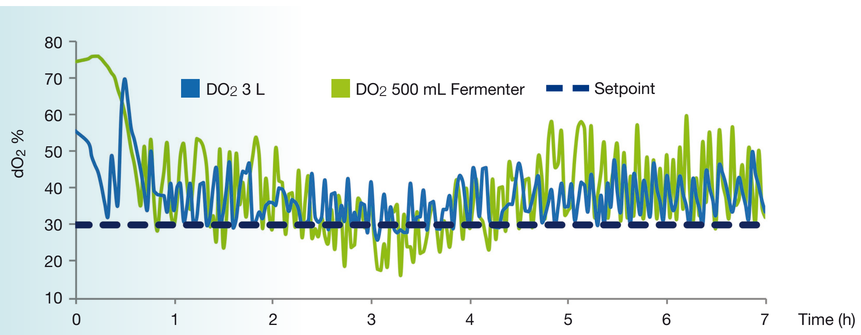

Figure 3A | Dissolved oxygen (air saturation %) in a 3 L bioreactor controlled with the ez-control and using air valve as actuator – green lines; Dissolved oxygen (air saturation %) in a 500 mL fermenter controlled with my-control and using air valve as actuator – blue lines.

The dO_2 was controlled during 7 h of batch fermentation at the set point of 30 % using air. The results from Figure 3A show a good control of the parameter above the set point as expected.
The comparison of the control between the two scales bioreactors and the two different controllers, allowed us to conclude that the ez-control and the my-control were both able to successfully control the parameters during the process. Furthermore, these results showed a same growth behavior of the culture in two different scales which reinforces the success of scalability between the 3 L and 500 mL bioreactors.

3B

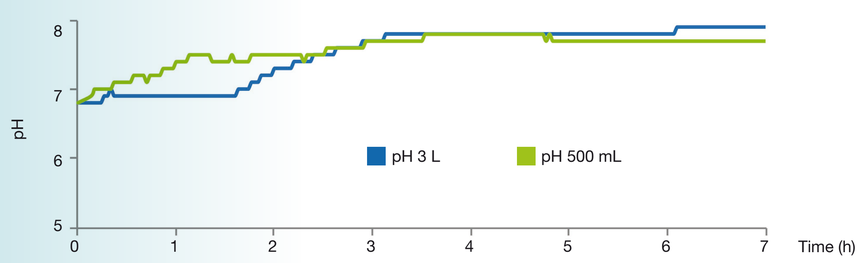

Figure 3B | pH in a 3 L fermenter controlled with the ez-control and using an alkali peristaltic pump as actuator – blue lines; pH in a 500 mL fermenter controlled with the ez-control and using an alkali peristaltic pump as actuator - green lines.

In Figure 3B is presented the pH profile along the fermentation. The set point for pH was 7 although, during the cultivation it was only used an actuator for upward control, meaning that base solution was added when the pH went below the set point which occurs due to the acid produced during bacterial growth [5]. The fact that it was not used an actuator for down control on the pH loop explains why the pH value is seen above the set point.
The proximity of the pH values observed during batch fermentation, specially when there was no control (above the set point) corroborates that both bioreactors enabled the same growth condition.

3C

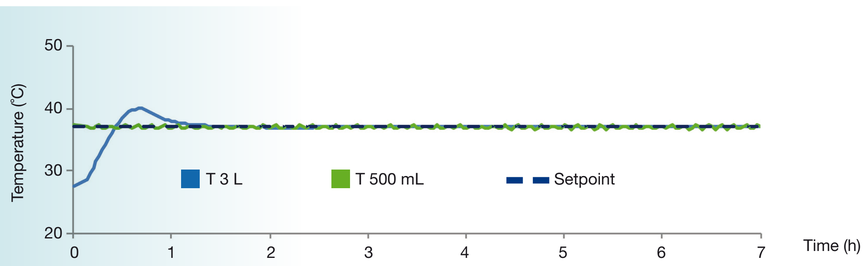

Figure 3C | Temperature in a 3 L fermenter using a cold finger (down control) and a heating blanked (up control), controlled with ez-control – blue line: Temperature in a 500 mL fermenter using a heating/cooling peltier (up and down control), controlled with my- control – green lines.

It is possible to observe in Figure 3C, that both systems were effective controlling the temperature at the set point of 37 °C. Even though different actuators were used - heating blanket (below set point) and the cold finger (above set point) for the 3 L bioreactor, while the 500 mL used a heating/cooling peltier element, both system presented an optimal control of temperature at the set point.
For the 3 L bioreactor it was observed an overshoot of the temperature value during the first hour of experiment. This overshoot is due to the PID values used for the control of the temperature. Initially there was a high output from the heating blanket and although it decreased with the proximity to the set point, it was not fast enough. Therefore, a change in the proportional value could have improved and/or prevent the initial overshoot of the temperature in the 3 L bioreactor.

Conclusions

- *E. coli* parallel fermentations were performed in 3 L and 500 mL bioreactors using the same conditions. The biomass study showed that both bioreactors allowed a similar growth rate. A final OD_{600} of 7.12 and 6.81 was achieved for the 3 L and 500 mL bioreactors, respectively with a difference of 4.4 % showing a very close final biomass achieved between the two bioreactors.
- The bioreactors used different controllers, Applikon's ez-control was used for the 3 L and my-control for the 500 mL. The inserts in the vessels and actuators for process control were the same for the two bioreactors (adjusted to scale), except for temperature control. The results showed a perfect control of the parameters dO_2, pH and temperature in both bioreactors and underlined that *E. coli* grew under the same conditions in the different scale bioreactors.
- With the present work, the scalability between Applikon's 3 L and 500 mL was proved and it was shown that both scales are reliable options for small-scale processes.

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